

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
5 April 2001 (05.04.2001)

PCT

(10) International Publication Number
WO 01/23429 A1

- (51) International Patent Classification?: C07K 14/725, 7/08, 16/28, C07H 21/04, A61K 38/10, 38/17, 39/395, A61P 37/08, 11/06
- (21) International Application Number: PCT/AU00/01182
- (22) International Filing Date:
22 September 2000 (22.09.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
PQ 3057 24 September 1999 (24.09.1999) AU
PQ 8964 24 July 2000 (24.07.2000) AU
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- (81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:
— With international search report.
— With (an) indication(s) in relation to deposited biological
material furnished under Rule 13bis separately from the
description.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: IMMUNOINTERACTIVE MOLECULES AND THEIR USES IN TREATING SUBJECTS SUFFERING FROM HEV B 5 ALLERGIES

(57) Abstract: The present invention relates generally to molecules such as peptides, polypeptides and proteins which interact immunologically with T lymphocytes in subjects having latex allergy and genetic sequences encoding same. These molecules are preferentially immunointeractive with T cells in subjects having a Hev b 5 allergy. The present invention also extends to antibodies, preferably monoclonal antibodies, directed to latex allergens and in particular to Hev b 5, and to the B cell epitopes recognised therein. The molecules of the present invention are useful in the development of diagnostic, therapeutic and prophylactic agents for conditions characterised by an aberrant, inappropriate or otherwise unwanted immune response to Hev b 5 or derivative or homologue thereof.

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Immunointeractive molecules and their uses in treating subjects suffering from Hev b 5 Allergies.**FIELD OF THE INVENTION**

The present invention relates generally to molecules such as peptides, polypeptides and proteins which interact immunologically with T lymphocytes in subjects having latex allergy and genetic sequences encoding same. These molecules are preferentially immunointeractive with T cells in subjects having a Hev b 5 allergy. The present invention also extends to antibodies, preferably monoclonal antibodies, directed to latex allergens and in particular to Hev b 5, and to the B cell epitopes recognised therein. The molecules of the present invention are useful in the development of diagnostic, therapeutic and prophylactic agents for conditions characterised by an aberrant, inappropriate or otherwise unwanted immune response to Hev b 5 or derivative or homologue thereof.

BACKGROUND OF THE INVENTION

Bibliographic details of the publications referred to by author in this specification are collected alphabetically at the end of the description.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

IgE mediated hypersensitivity to latex has emerged as a serious occupational health problem since the introduction of universal precautions in the mid 1980s (Slater, 1994; Sussman *et al.*, 1991). The use of latex gloves for barrier protection may lead to sensitisation, especially of health care workers (HCW), to protein allergens present in the natural rubber latex. The prevalence of latex sensitisation amongst HCW has been reported from 8.2-22% (Grzybowski *et al.*, 1996; Kibby and Akl, 1997; Liss *et al.*, 1997; Brown *et al.*, 1988; Douglas *et al.*, 1997). Allergic reactions to latex range from urticaria, rhinoconjunctivitis, asthma (Brugnami *et al.*, 1995), and angioedema to severe generalised

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anaphylaxis in some cases (Ownby *et al.*, 1991). Since the only form of treatment available at present is allergen avoidance and symptomatic relief, there is an urgent need for the development of specific immunotherapy for this condition. Thus, the immunological characterisation of latex allergens is an important breakthrough in the development of rational curative treatments for latex allergy.

Several allergens from the rubber plant *Hevea brasiliensis* have been identified (Chen *et al.*, 1996; Yeang *et al.*, 1996; Sowka *et al.*, 1998; Scheiner *et al.*, 1999; Kostyal *et al.*, 1998; Akasawa *et al.*, 1996; Slater *et al.*, 1996; Chen *et al.*, 1998; Posch *et al.*, 1998). Based on IgE binding studies, certain latex allergens seem to be preferentially recognised by particular risk groups (Hev b 1 and 3 by children with spina bifida and Hev b 5 and 6.02 by HCW (Breitender, 1998)). Hev b 5, a highly acidic and proline rich protein with a predicted predominantly random secondary structure, has been shown to react with IgE from 92% of HCW and 56% of spina bifida patients with latex allergy (Slater *et al.*, 1996). Thus Hev b 5 has been identified as a major allergen in natural rubber latex. Interestingly, the amino acid sequence of Hev b 5 shows 46% identity to another acidic protein identified in kiwi fruit (*Actinidia deliciosa*) (Slater *et al.*, 1996) and this may provide a molecular explanation for the high frequency of fruit hypersensitivity seen in latex allergic patients (Brehler *et al.*, 1997).

The production of allergen specific IgE by B cells and release of inflammatory mediators by mast cells and eosinophils result in the effector response of allergic disease. However, it is well established that these events are orchestrated by allergen-specific CD4⁺ T cells with a Th₂-type cytokine profile. T cell reactive determinants have been reported for another major latex allergen, Hev b 1 (Raulf-Heimsoth, 1998), but not for Hev b 5. Thus, characterisation of the immune response to Hev b 5 is critical in the development of specific diagnostic and immunotherapeutic methodology.

In work leading up to the present invention, the inventors have identified the human T cell epitopes of the latex allergen, Hev b 5. Although murine T cell epitopes of Hev b 5 have previously been identified in BALB/c mice, there is not necessarily a correlation between

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species in terms of the sharing of T or B cell epitopic regions on homologous molecules. In this regard, the inventors have confirmed that the immunodominant human Hev b 5 T cell epitopes which they have identified, and which are disclosed herein, do not correspond to the dominant murine T cell Hev b 5 epitopes. This variation between murine and human immunodominance demonstrates the difficulties associated with elucidating aspects of immune response mechanisms, such as the identification of epitopes, and highlights the novel and surprising results obtained by the inventors. The inventors have also developed monoclonal antibodies directed to Hev b 5 thereby facilitating the identification of Hev b 5 B cell epitopes. The identification of Hev b 5 T and B cell epitopes and the production of monoclonal antibodies to Hev b 5 now facilitates the development of molecules and methodology for the diagnosis and treatment of conditions characterised by the aberrant, inappropriate or otherwise unwanted immune response to Hev b 5 or derivative or homologue thereof such as latex allergy or fruit hypersensitivity.

SUMMARY OF THE INVENTION

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The subject specification contains amino acid sequence information prepared using the programme PatentIn Version 2.0, presented herein after the bibliography. Each amino acid sequence is identified in the sequence listing by the numeric indicator <210> followed by the sequence identifier (eg. <210>1, <210>2, etc). The length, type of sequence (protein (PRT), etc) and source organism for each amino acid sequence is indicated by information provided in the numeric indicator fields <211>, <212> and <213>, respectively. Amino acid sequences referred to in the specification are defined by the information provided in numeric indicator field <400> followed by the sequence identifier (eg. <400>1, <400>2, etc).

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One aspect of the present invention provides an isolated peptide of the formula:



wherein:

X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;

X_2 is any amino acid sequence derived from or homologous to Hev b 5;

and wherein said peptide molecule is capable of interacting with T cells and modifying T cell function when incubated with cells from subjects having a condition characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5 or a derivative, homologue, analogue, mutant, chemical equivalent or mimetic of said peptide.

The present invention therefore more particularly provides an isolated peptide of the formula:



wherein:

X_1 X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;

X_2 is an amino acid sequence of from 5 to 100 residues derived from, homologous to or contiguous with amino acids 1-151 inclusive or derivatives thereof of Hev b 5;

and wherein said peptide molecule is capable of interacting with T cells and modifying T cell function when incubated with cells from subjects having a condition characterised by

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an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5 or a derivative, homologue, mutant, chemical equivalent or mimetic of said peptide.

Still more particularly the present invention provides an isolated peptide of the formula:



wherein

X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;

X_2 is an amino acid sequence of from 5 to 100 residues derived from, homologous to or contiguous with amino acids 37-74 or 109-146 inclusive or derivatives thereof of Hev b 5;

and wherein said peptide molecule is capable of interacting with T cells and modifying T cell function when incubated with cells from subjects having a condition characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5 or a derivative, homologue, analogue, mutant, chemical equivalent or mimetic of said peptide.

Still more particularly, X_2 is any amino acid sequence of from 5 to 100 residues derived from, homologous to or contiguous with amino acids 1-20, 19-110 and/or 109-146 inclusive or derivatives thereof of Hev b 5.

Yet more particularly, X_2 is any amino acid sequence of from 5 to 100 residues derived from homologous to or contiguous with amino acids 37-56, 46-65, 55-74, 109-128 and/or 127-146 inclusive or derivatives thereof of Hev b 5.

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Most particularly, X₂ is any amino acid sequence of from 5 to 100 residues derived from, homologous to or contiguous with amino acids 46-65 and/or 109-128 inclusive or derivatives thereof of Hev b 5.

Still more particularly, X₂ is any amino acid sequence of from 5 to 100 residues derived from, homologous to or contiguous with amino acids 51-60 inclusive or derivatives thereof of Hev b 5.

In a particularly preferred embodiment, X₂ comprises a sequence of at least 5 amino acids derived from one or more of the following amino acid sequences:

ASEQETADATPEKEEPTAAP <400>6

TPEKEEPTAAPAEPEAPE <400>7

APAEPEAPAPETEKAEVEK <400>8

TKETETEAPAAPAEGEKPAE <400>14

AEEKPPITEAAETATTEVPV <400>16

More preferably, X₂ comprises a sequence of at least 5 amino acids derived from one or more of <400>7 or <400>14.

Still more preferably, X₂ comprises a sequence of at least 5 amino acids derived from <400>7.

Most preferably, X₂ comprises a sequence of at least 5 amino acids derived from the following amino acid sequence:

EPTAAPAEPE <400>42.

Another aspect of the present invention provides an isolated peptide comprising any amino acid sequence derived from or homologous to Hev b 5 wherein said peptide molecule is capable of interacting with T cells and modifying T cell function when incubated with cells

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from subjects having a condition characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5 or a derivative, homologue, analogue, mutant, chemical equivalent or mimetic of said peptide.

Yet another aspect of the present invention provides an isolated peptide comprising an amino acid sequence of from 5-100 residues derived from, homologues to or contiguous with amino acids 1-151 inclusive or derivatives thereof of Hev b 5 wherein said peptide molecule is capable of interacting with T cells and modifying T cell function when incubated with cells from subjects having a condition characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5 or a derivative, homologue, analogue, mutant, chemical equivalent or mimetic of said peptide.

In one preferred embodiment said amino acid sequence is derived from, homologous to or contiguous with amino acids 37-74 or 109-146 inclusive or derivatives thereof of Hev b 5.

In another preferred embodiment said amino acid sequence is derived from, homologous to or contiguous with amino acids 1-20, 19-110 and/or 109-146 inclusive or derivatives thereof of Hev b 5.

In yet another preferred embodiment said amino acid sequence is derived from, homologous to or contiguous with amino acids 37-56, 46-65, 55-74, 109-128 and/or 127-146 inclusive or derivatives thereof of Hev b 5.

More preferably, said amino acid sequence is derived from, homologous to or contiguous with amino acids 46-65 and/or 109-128, and most preferably amino acids 51-60, inclusive or derivatives thereof of Hev b 5.

In another aspect said amino acid sequence comprises a sequence of at least 5 amino acids derived from one or more of the following amino acid sequences:

ASEQETADATPEKEEPTAAP <400>6

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TPEKEEPTAAPAEPEAPAE <400>7

APAEPEAPAPETEKAEVEK <400>8

TKETETEAPAAPAEGEKPAE <400>14

AEEKPITEAAETATTEVPV <400>16

According to this aspect, said amino acid sequence preferably comprises a sequence of at least 5 amino acids derived from one or more of <400>7 or <400>14.

Still more preferably, said amino acid sequence comprises a sequence of at least 5 amino acids derived from <400>7.

Most preferably, said amino acid sequence comprises a sequence of at least 5 amino acids derived from the following amino acid sequence:

EPTAAPAEPE <400>42.

Another aspect of the present invention is directed to antibodies to Hev b 5 including catalytic antibodies or derivatives, homologues, analogues, mutants, chemical equivalents or mimetics of said antibodies.

In a preferred embodiment the subject antibody is 1C10, 6F6, 3G3 or 6A10 and, more particularly, 1C10, 6F6 or 3G3 or derivative, homologue, analogue, chemical equivalent, mutant or mimetic thereof.

In another aspect there is provided an isolated peptide of the formula:

$$X_1 X_2 X_3$$

wherein

X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;

X_2 is an amino acid sequence derived from, or homologous to Hev b 5

and wherein said peptide molecule is capable of interacting with antibody from subjects having a condition characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5 or a derivative, homologue, analogue, mutant, chemical equivalent or mimetic of said peptide.

Preferably, said condition is latex hypersensitivity and said antibodies are of the IgE isotype.

More particularly the present invention provides an isolated peptide of the formula:

$X_1 X_2 X_3$

wherein

X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;

X_2 is any amino acid sequence derived from, or homologous to Hev b 5

and wherein said peptide molecule is capable of interacting with antibodies 1C10, 6F6, 3G3 or 6A10 or a derivative, homologue, analogue, mutant, chemical equivalent or mimetic of said peptide.

In another aspect, the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding the peptides as hereinbefore defined or a derivative, homologue or analogue thereof.

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In yet another aspect the present invention provides a method for the treatment and/or prophylaxis of a condition in the subject, which condition is characterised by the aberrant, unwanted or otherwise inappropriate immune response to Hev b 5, said method comprising administering to said subject an effective amount of a peptide and/or antibody as hereinbefore defined for a time and under conditions sufficient to remove or reduce the presence or function in said subject of T cells and/or antibodies directed to said Hev b 5.

Still another aspect of the present invention contemplates the use of an agent as hereinbefore defined in the manufacture of a medicament for the treatment of a condition in a mammal, which condition is characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5.

In yet another further aspect, the present invention contemplates a pharmaceutical composition comprising an agent as hereinbefore defined and one or more pharmaceutically acceptable carriers and/or diluents. Said agents are referred to as the active ingredients.

Yet another aspect of the present invention relates to agents, as hereinbefore defined, when used in the method of the present invention.

Still yet another aspect of the present invention is directed to a method of diagnosing or monitoring a condition in a mammal, which condition is characterised by an aberrant, unwanted or inappropriate response to Hev b 5, said method comprising screening for Hev b 5 reactive T cells and/or antibodies utilising a peptide as hereinbefore defined.

Still another aspect of the present invention is directed to a method of qualitatively and/or quantitatively detecting Hev b 5, or peptides thereof, in a sample said method comprising screening for said Hev b 5 or peptides thereof utilising an antibody as hereinbefore defined.

In a further aspect the present invention provides diagnostic kits for use in the diagnostic methodology hereinbefore defined.

Single and three letter abbreviations used throughout the specification are defined in Table 1.

TABLE 1
Single and three letter amino acid abbreviations

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of the primary amino acid sequences of overlapping synthetic peptides, spanning the entire length of the Hev b 5 molecule. Amino acid residues are indicated with the single letter code. All peptides are 20 mers with 11 amino acid overlap except for the last two peptide that overlap by 15 residues.

Figure 2 is an image of dot immunoblot analysis of serum IgE reactivity with latex allergens for the latex allergic patients 1-6.

Figure 3 is a graphical representation of the proliferation of (A) PBMC and (B) latex specific T cell line of patient 2 in response to latex allergens. (A) PBMC proliferative response to LAL (0.03-100 µg/ml), rHev b 5/MBP (5-40 µg/ml), MBP (5-40 µg/ml) was assessed by ^3H thymidine incorporation. Tetanus Toxoid (tet-tox) (0.3 Lf/ml) and Lymphocult-T (10 IU/ml) were included as controls. Results are expressed as cpm \pm SD of triplicate cultures. Cells alone in the absence of antigen were included to assess background levels of cell proliferation. (B) T cell line proliferation in the presence of Hev b 5 peptides (10,30 µg/ml), LAL (3-100 µg/ml), rHev b 5/MBP (10 µg/ml), MBP (10 µg/ml), PHA (3 µg/ml) and Lymphocult-T (10 IU/ml) was assessed by ^3H -thymidine incorporation. T+APC alone, T alone and APC alone were included to assess background levels of cell proliferation.

Figure 4 is a graphical representation of the (A) Proliferation and (B) IL-5 production by latex specific T cell line of latex allergic patient 4. (A) Proliferation of T cells in response to Hev b 5 peptides (30, 10 µg/ml), LAL (100, 30, 10, 3 µg/ml) and PHA (3 µg/ml) assessed by ^3H thymidine incorporation (values shown are averages of triplicate samples with SD indicated). Background level of cell proliferation (T+APC) is indicated by the horizontal line at 1000 cpm. (B) Production of IL-5 in the culture supernatants determined by ELISA. Only the indicated peptides were tested for their ability to stimulate T cell production of IL-5. NT indicates peptides that were not tested in the IL-5 assay. Values indicate averages of duplicate samples.

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Figure 5 is a graphical representation of the comparison of human and murine T cell determinants of Hev b 5. Latex allergic patient (1-5) T cell stimulation indices ≥ 2.5 in response to Hev b 5 peptide stimulation are shown by the hatched bars. Previously identified murine T cell epitopes³⁰ are shown in shading.

Figure 6 is a graphical representation of the immune response of mouse number 3 during the immunisation schedule with rHev b5/MBP.

Figure 7 is a graphical representation of an ELISA of hybridoma supernatants.

Figure 8 is an image of a Western blot of a rHev b 5 fusion protein digest.

Figure 9 is an image of a Western blot of NAL.

Figure 10 is a graphical representation of the determination of the minimal epitope of Hev b 5 p(46-65). A three week low ammoniated latex (LAL)-specific T cell line was stimulated with the Hev b 5 p(46-65) truncated peptides at two concentrations, 10 $\mu\text{g/ml}$ and 30 $\mu\text{g/ml}$. Proliferation was assessed at 72 hours by ^3H -thymidine incorporation in counts per minute (A). Levels of IL-5 (B) and IFN- γ (C) in culture supernatants were measured at 48 hours using a commercially available sandwich ELISA kit.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention is predicated, in part, on the identification of Hev b 5 T and B cell epitopic regions and the development of monoclonal antibodies to Hev b 5. The identification of immunodominant epitopes of Hev b 5 has enabled the improvement of diagnostic methodology and the development of therapeutic and prophylactic compositions and treatment approaches for conditions such as, but not limited to, latex allergy.

In accordance with the present invention, overlapping peptides were synthesised based on the Hev b 5 amino acid sequence disclosed in <400>1. The T cell immunoreactivity of these peptides is identified in accordance with the present invention on the basis of interactivity of peripheral blood cells or T cells obtained from the peripheral blood of subjects with latex hypersensitivity. The identification of Hev b 5 B cell epitopes has been facilitated via the generation of a panel of monoclonal antibodies directed to Hev b 5. The identification and generation of those molecules thereby form the basis for a new range of diagnostic, therapeutic and prophylactic reagents and procedures.

Accordingly, one aspect of the present invention provides an isolated peptide of the formula:



wherein:

X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;

X_2 is any amino acid sequence derived from or homologous to Hev b 5;

and wherein said peptide molecule is capable of interacting with T cells and modifying T cell function when incubated with cells from subjects having a condition characterised by

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an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5 or a derivative, homologue, analogue, mutant, chemical equivalent or mimetic of said peptide.

Hev b 5 is a protein which has been identified in the rubber plant *Hevea brasiliensis*. It is a highly acidic and proline rich protein with a predicted predominantly random secondary structure. It has also been shown to share homology with acidic proteins found in fruit, in particular, an acidic protein identified in kiwi fruit (*Actinidia deliciosa*) (Slater *et al.*, 1996). Accordingly, reference to "Hev b 5" should be understood as including reference to all forms of Hev b 5 or derivatives, mutants, homologues, analogues, equivalents or mimetics thereof. This includes, for example, all protein forms of Hev b 5 or its functional equivalent or derivative including, for example, any isoforms which may arise from alternative splicing of Hev b 5 mRNA. It includes reference to mutants, polymorphic variants or homologues of Hev b 5, such as the homologous kiwi fruit protein detailed above. It also includes reference to analogues or equivalents of Hev b 5 such as may occur where a product which naturally comprises Hev b 5 is synthetically generated for the purpose of generating a product such as gloves. The present invention thereby provides epitopes and methods for their use in the diagnosis and treatment of any condition characterised by hypersensitivity to a Hev b 5 or Hev b 5-like molecule such as latex allergy or a fruit allergy. Preferably, said Hev b 5 comprises the sequence set forth in <400>1 or is a derivative, homologue, analogue, chemical equivalent, mutant or mimetic of said sequence.

The present invention therefore more particularly provides an isolated peptide of the formula:



wherein:

X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;

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X₂ is an amino acid sequence of from 5 to 100 residues derived from, homologous to or contiguous with amino acids 1-151 inclusive or derivatives thereof of Hev b 5;

and wherein said peptide molecule is capable of interacting with T cells and modifying T cell function when incubated with cells from subjects having a condition characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5 or a derivative, homologue, mutant, chemical equivalent or mimetic of said peptide.

Still more particularly the present invention provides an isolated peptide of the formula:



wherein

X₁ and X₃ may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;

X₂ is an amino acid sequence of from 5 to 100 residues derived from, homologous to or contiguous with amino acids 37-74 or 109-146 inclusive or derivatives thereof of Hev b 5;

and wherein said peptide molecule is capable of interacting with T cells and modifying T cell function when incubated with cells from subjects having a condition characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5 or a derivative, homologue, analogue, mutant, chemical equivalent or mimetic of said peptide.

Still more particularly, X₂ is any amino acid sequence of from 5 to 100 residues derived from, homologous to or contiguous with amino acids 1-20, 19-110 and/or 109-146 inclusive or derivatives thereof of Hev b 5.

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Yet more particularly, X_2 is any amino acid sequence of from 5 to 100 residues derived from homologous to or contiguous with amino acids 37-56, 46-65, 55-74, 109-128 and/or 127-146 inclusive or derivatives thereof of Hev b 5.

Most particularly, X_2 is any amino acid sequence of from 5 to 100 residues derived from, homologous to or contiguous with amino acids 46-65 and/or 109-128 inclusive or derivatives thereof of Hev b 5.

Still more particularly, X_2 is any amino acid sequence of from 5 to 100 residues derived from, homologous to or contiguous with amino acids 51-60 inclusive or derivatives thereof of Hev b 5.

Reference to "T cells" should be understood as a reference to any cell comprising a T cell receptor. In this regard, the T cell receptor may comprise any one or more of the α , β , γ or δ chains. The present invention is not intended to be limited to any particular functional sub-class of T cells although in a preferred embodiment the subject T cell is a T helper cell and still more preferably a Th2-type cell, predominantly. In this regard, reference to "modifying T cell function" should be understood as a reference to modifying any one or more functions which a T cell is capable of performing. For example, the subject function may be proliferation, differentiation or other form of cellular functional activity such as the production of cytokines. Preferably, the subject functional activity is proliferation.

In terms of modifying the function of T cells from subjects having a condition characterised by an aberrant, unwanted or inappropriate immune response to Hev b 5, it should be understood that this is not necessarily a reference to modifying the function of all the T cells in a given sample but is likely, in fact, to reflect the modification or functioning of only some of the T cells in the sample. For example, only a portion of the T helper cells in a given T cell sample may functionally respond to contact with the subject peptide. Such a partial response should be understood to fall within the scope of the present invention. It should also be understood that the T cells which are derived from the subject may be freshly harvested T cells or they may have undergone some form of *in vitro* or *in*

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vivo manipulation prior to testing. For example, T cell lines may have been generated from the cell sample and it is these T cell lines which then form the subject derived T cell population which is tested in accordance with the present invention. To the extent that the subject functional activity is T cell proliferation, the T cell proliferation assay is preferably performed as disclosed herein. Still more preferably, the subject modification of T cell function is the induction of a proliferation index of ≥ 2.5 .

Reference to an "aberrant, unwanted or otherwise inappropriate" immune response should be understood as a reference to any form of physiological activity which involves the activation and/or functioning of one or more immune cells where that activity is inappropriate in that it is of an inappropriate type or proceeds to an inappropriate degree. It may be aberrant in that according to known immunological principals it either should not occur when it does so or else should occur when it does not do so. In another example, the immune response may be inappropriate in that it is a physiologically normal response but which is unnecessary and/or unwanted, such as occurs with respect to type-I hypersensitivity responses to innocuous allergens. Preferably said immune response is latex hypersensitivity.

By "latex hypersensitivity" it should be understood to mean the exhibition of clinical symptoms of IgE mediated latex hypersensitivity as tested via latex specific serum IgE which is measured using the Kallestad Allercoat EAST System (Sanofi-Pasteur Diagnostics, USA). In accordance with this test, the latex EAST score in non-allergic individuals is 0/4. Accordingly any subject exhibiting a latex EAST score greater than 0/4 should be understood as a subject exhibiting latex hypersensitivity within the context of the present invention.

In a preferred embodiment, X₂ comprises not less than about 5 and not greater than about 50 amino acid residues, more preferably not less than about 5 and not greater than about 30 amino acid residues and even more preferably not less than about 5 and not greater than about 20.

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In a particularly preferred embodiment, X_2 comprises a sequence of at least 5 amino acids derived from one or more of the following amino acid sequences:

ASEQETADATPEKEEPTAAP <400>6

TPEKEEPTAAPAEPEAPAE <400>7

APAEPEAPAPETEKAEVEK <400>8

TKETETEAPAAPAEGEKPAE <400>14

AEEKPITEAAETATTEVPV <400>16

More preferably, X_2 comprises a sequence of at least 5 amino acids derived from one or more of <400>7 or <400>14.

Still more preferably, X_2 comprises a sequence of at least 5 amino acids derived from <400>7.

Most preferably, X_2 comprises a sequence of at least 5 amino acids derived from the following amino acid sequence:

EPTAAPAEPE <400>42.

Reference to a "peptide" includes reference to a peptide, polypeptide or protein or parts thereof. The peptide may be glycosylated or unglycosylated and/or may contain a range of other molecules fused, linked, bound or otherwise associated to the protein such as amino acids, lipids, carbohydrates or other peptides, polypeptides or proteins. Reference hereinafter to a "peptide" includes a peptide comprising a sequence of amino acids as well as a peptide associated with other molecules such as amino acids, lipids, carbohydrates or other peptides, polypeptides or proteins.

"Derivatives" include fragments, parts, portions and variants from natural, synthetic or recombinant sources including fusion proteins. Parts or fragments include, for example, active regions of the subject peptide. Derivatives may be derived from insertion, deletion or substitution of amino acids. Amino acid insertional derivatives include amino and/or

carboxylic terminal fusions as well as intrasequence insertions of single or multiple amino acids. Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterized by the removal of one or more amino acids from the sequence.

Substitutional amino acid variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. An example of substitutional amino acid variants are conservative amino acid substitutions. Conservative amino acid substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine and leucine; aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine. Additions to amino acid sequences include fusions with other peptides, polypeptides or proteins.

Chemical and functional equivalents of the subject peptide should be understood as molecules exhibiting any one or more of the functional activities of these molecules and may be derived from any source such as being chemically synthesized or identified via screening processes such as natural product screening.

Homologues include peptides derived from species other than *Hevea brasiliensis*, such as peptides derived from kiwi fruit.

The derivatives include fragments having particular epitopes or parts of the entire protein fused to peptides, polypeptides or other proteinaceous or non-proteinaceous molecules.

Analogues contemplated herein include, but are not limited to, modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecules or their analogues.

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Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with NaBH_4 ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with NaBH_4 .

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carboethoxylation with diethylpyrocarbonate.

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Examples of incorporating unnatural amino acids and derivatives during protein synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acids contemplated herein is shown in Table 2.

TABLE 2

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α -aminobutyric acid	Abu	L-N-methylalanine	Nmala
α -amino- α -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
aminocyclopropane- carboxylate	Cpro	L-N-methylasparagine	Nmasn
aminoisobutyric acid	Aib	L-N-methylaspartic acid	Nmasp
aminonorbornyl- carboxylate	Norb	L-N-methylcysteine	Nmcys
cyclohexylalanine	Chexa	L-N-methylglutamine	Nmgln
cyclopentylalanine	Cpen	L-N-methylglutamic acid	Nmglu
D-alanine	Dal	L-N-methylhistidine	Nmhis
D-arginine	Darg	L-N-methylisoleucine	Nmile
D-aspartic acid	Das	L-N-methylleucine	Nmleu
D-cysteine	Dcys	L-N-methyllysine	Nmlys
D-glutamine	Dgln	L-N-methylmethionine	Nmmet
D-glutamic acid	Dglu	L-N-methylnorleucine	Nmnle
D-histidine	Dhis	L-N-methylnorvaline	Nmnva
D-isoleucine	Dile	L-N-methylornithine	Nmorn
D-leucine	Dleu	L-N-methylphenylalanine	Nmphe
D-lysine	Dlys	L-N-methylproline	Nmpro
D-methionine	Dmet	L-N-methylserine	Nmser
D-ornithine	Dorn	L-N-methylthreonine	Nmthr
D-phenylalanine	Dphe	L-N-methyltryptophan	Nmtrp
D-proline	Dpro	L-N-methyltyrosine	Nmtyr
D-serine	Dser	L-N-methylvaline	Nmval
D-threonine	Dthr	L-N-methylethylglycine	Nmetg
D-tryptophan	Dtrp	L-N-methyl-t-butylglycine	Nmtbug
D-tyrosine	Dtyr	L-norleucine	Nle
D-valine	Dval	L-norvaline	Nva
		α -methyl-aminoisobutyrate	Maib
		α -methyl- γ -aminobutyrate	Mgab

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D- α -methylalanine	Dmala	α -methylcyclohexylalanine	Mchexa
D- α -methylarginine	Dmarg	α -methylcycopentylalanine	Mcpen
D- α -methylasparagine	Dmasn	α -methyl- α -naphthylalanine	Manap
D- α -methylaspartate	Dmasp	α -methylpenicillamine	Mpen
D- α -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D- α -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D- α -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
D- α -methylisoleucine	Dmile	N-amino- α -methylbutyrate	Nmaabu
D- α -methylleucine	Dmleu	α -naphthylalanine	Anap
D- α -methyllysine	Dmlys	N-benzylglycine	Nphe
D- α -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D- α -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D- α -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D- α -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D- α -methylserine	Dmser	N-cyclobutylglycine	Ncbut
D- α -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D- α -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D- α -methyltyrosine	Dmtty	N-cyclodecylglycine	Ncdec
D- α -methylvaline	Dmval	N-cylcododecylglycine	Ncdod
D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylglutamine	Dnmglu	N-(3-guanidinopropyl)glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylethyl)glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl- γ -aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen

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N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpgro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyl- α -naphthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ -aminobutyric acid	Gabu	N-(<i>p</i> -hydroxyphenyl)glycine	Nhtyr
L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L- α -methylalanine	Mala
L- α -methylarginine	Marg	L- α -methylasparagine	Masn
L- α -methylaspartate	Masp	L- α -methyl- <i>t</i> -butylglycine	Mtbug
L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
L- α -methylglutamine	Mgln	L- α -methylglutamate	Mglu
L- α -methylhistidine	Mhis	L- α -methylhomophenylalanine	Mhphe
L- α -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L- α -methylleucine	Mleu	L- α -methyllysine	Mlys
L- α -methylmethionine	Mmet	L- α -methylnorleucine	Mnle
L- α -methylnorvaline	Mnva	L- α -methylornithine	Morn
L- α -methylphenylalanine	Mphe	L- α -methylproline	Mpro
L- α -methylserine	Mser	L- α -methylthreonine	Mthr
L- α -methyltryptophan	Mtrp	L- α -methyltyrosine	Mtyr
L- α -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhph
N-(N-(2,2-diphenylethyl)	Nnbhm	N-(N-(3,3-diphenylpropyl)	Nnbhe
carbonylmethyl)glycine		carbonylmethyl)glycine	
l-carboxy-1-(2,2-diphenyl-Nmbc			
ethylamino)cyclopropane			

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having (CH₂)_n spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-

bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety.

It is possible to modify the structure of a peptide according to the invention for various purposes such as for increasing solubility, enhancing therapeutic or preventative efficacy, enhancing stability or increasing resistance to proteolytic degradation. A modified peptide may be produced in which the amino acid sequence has been altered, such as by amino acid substitution, deletion or addition, to modify immunogenicity and/or reduce allergenicity. Similarly components may be added to peptides of the invention to produce the same result.

For example, a peptide can be modified so that it exhibits the ability to induce T cell energy. In this instance, critical binding residues for the T cell receptor can be determined using known techniques (for example substitution of each residue and determination of the presence or absence of T cell reactivity). In one example, those residues shown to be essential to interact with the T cell receptor can be modified by replacing the essential amino acid with another, preferably similar amino acid residue (a conservative substitution) whose presence is shown to alter T cell reactivity or T cell functioning. In addition, those amino acid residues which are not essential for T cell receptor interaction can be modified by being replaced by another amino acid whose incorporation may then alter T cell reactivity or T cell functioning but does not, for example, eliminate binding to relevant MHC proteins.

Such modifications will result in the production of molecules falling within the scope of "mutants" of the subject peptide as herein defined. "Mutants" should be understood as a reference to peptides which exhibit one or more structural features or functional activities which are distinct from those exhibited by the non-mutated peptide counterpart. Peptides of the invention may also be modified to incorporate one or more polymorphisms resulting from natural allelic variation and D-amino acids, non-natural amino acids or amino acid analogues may be substituted into the peptides to produce modified peptides which fall within the scope of the invention. Peptides may also be modified by conjugation

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with polyethylene glycol (PEG) by known techniques. Reporter groups may also be added to facilitate purification and potentially increase solubility of the peptides according to the invention. Other well known types of modification including insertion of specific endoprotease cleavage sites, addition of functional groups or replacement of hydrophobic residues with less hydrophobic residues as well as site-directed mutagenesis of DNA encoding the peptides of the invention may also be used to introduce modifications which could be useful for a wide range of purposes. The various modifications to peptides according to the invention which have been mentioned above are mentioned by way of example only and are merely intended to be indicative of the broad range of modifications which can be effected.

Another aspect of the present invention provides an isolated peptide comprising any amino acid sequence derived from or homologues to Hev b 5 wherein said peptide molecule is capable of interacting with T cells and modifying T cell function when incubated with cells from subjects having a condition characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5 or a derivative, homologue, analogue, mutant, chemical equivalent or mimetic of said peptide.

More particularly, the present invention provides an isolated peptide comprising an amino acid sequence of from 5-100 residues derived from, homologues to or contiguous with amino acids 1-151 inclusive or derivatives thereof of Hev b 5 wherein said peptide molecule is capable of interacting with T cells and modifying T cell function when incubated with cells from subjects having a condition characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5 or a derivative, homologue, analogue, mutant, chemical equivalent or mimetic of said peptide.

In one preferred embodiment said amino acid sequence is derived from, homologous to or contiguous with amino acids 37-74 or 109-146 inclusive or derivatives thereof of Hev b 5.

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In another preferred embodiment said amino acid sequence is derived from, homologous to or contiguous with amino acids 1-20, 19-110 and/or 109-146 inclusive or derivatives thereof of Hev b 5.

In yet another preferred embodiment said amino acid sequence is derived from, homologous to or contiguous with amino acids 37-56, 46-65, 55-74, 109-128 and/or 127-146 inclusive or derivatives thereof of Hev b 5.

More preferably, said amino acid sequence is derived from, homologous to or contiguous with amino acids 46-65 and/or 109-128, and most preferably amino acids 51-60, inclusive or derivatives thereof of Hev b 5.

In another aspect said amino acid sequence comprises a sequence of at least 5 amino acids derived from one or more of the following amino acid sequences:

ASEQETADATPEKEEPTAAP <400>6

TPEKEEPTAAPAEPEAPAE <400>7

APAEPEAPAPETEKAEVEK <400>8

TKETETEAPAAPAEGEKPAE <400>14

AEEKPITEAAETATTEVPV <400>16

According to this aspect, said amino acid sequence preferably comprises a sequence of at least 5 amino acids derived from one or more of <400>7 or <400>14.

Still more preferably, said amino acid sequence comprises a sequence of at least 5 amino acids derived from <400>7.

Most preferably, said amino acid sequence comprises a sequence of at least 5 amino acids derived from the following amino acid sequence:

EPTAAPAEPE <400>42.

The peptides of the present invention may be prepared by recombinant or chemical synthetic means. According to a preferred aspect of the present invention, there is provided a recombinant peptide which is preferentially immunologically reactive with T cells from individuals with latex hypersensitivity, which is expressed by the expression of a host cell transformed with a vector coding for the peptide sequence of the present invention. The peptide may be fused to another peptide, polypeptide or protein. Alternatively, the peptide may be prepared by chemical synthetic techniques, such as by the Merrifield solid phase synthesis procedure. Furthermore, although synthetic peptides of the formula given above represent a preferred embodiment, the present invention also extends to biologically pure preparations of the naturally occurring peptides or fragments thereof. By "biologically pure" is meant a preparation comprising at least about 60%, preferably at least about 70%, or preferably at least about 80% and still more preferably at least about 90% or greater as determined by weight, activity or other suitable means.

Still another aspect of the present invention is directed to antibodies to Hev b 5 including catalytic antibodies or derivatives, homologues, analogues, mutants, chemical equivalents or mimetics of said antibodies. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to Hev b 5 or may be specifically raised to Hev b 5. In the case of the latter, Hev b 5 may first need to be associated with a carrier molecule. The antibodies and/or recombinant Hev b 5 of the present invention are particularly useful as therapeutic or diagnostic agents. Alternatively, fragments of antibodies may be used such as Fab fragments or Fab₂ fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies. Hev b 5 can also be used to screen for naturally occurring antibodies to Hev b 5.

Both polyclonal and monoclonal antibodies are obtainable by immunization with Hev b 5 or derivative, homologue, analogue, mutant, chemical equivalent or mimetic thereof and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared

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by injection of a suitable laboratory animal with an effective amount of Hev b 5, or antigenic parts thereof, collecting serum from the animal, and isolating specific sera by any of the known immunoadsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art. (See, for example Douillard and Hoffman, Basic Facts about Hybridomas, in *Compendium of Immunology* Vol II, ed. by Schwartz, 1981; Kohler and Milstein, *Nature* 256: 495-499, 1975; *European Journal of Immunology* 6: 511-519, 1976).

Preferably, the antibody of the present invention specifically binds Hev b 5 or derivative, homologue, analogue, mutant, chemical equivalent or mimetic thereof. By "specifically binds" is meant high avidity and/or high affinity binding of an antibody to a specific antigen. Antibody binding to its epitope on this specific antigen is stronger than binding of the same antibody to any other epitope, particularly those that may be present in molecules in association with, or in the same sample, as the specific antigen of interest. Antibodies that bind specifically to a polypeptide of interest may be capable of binding other polypeptides at a weak, yet detectable, level (e.g., 10% or less of the binding shown to the polypeptide of interest). Such weak binding, or background binding, is readily discernible from the specific antibody binding to the polypeptide of interest, e.g. by use of appropriate controls.

In a preferred embodiment the subject antibody is 1C10, 6F6, 3G3 or 6A10 and, more particularly, 1C10, 6F6 or 3G3 or derivative, homologue, analogue, chemical equivalent, mutant or mimetic thereof.

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Hybridoma cell lines secreting 1C10, 6F6, 3G3 and 6A10 were deposited with ECACC on 19 September, 2000.

The development of monoclonal antibodies to Hev b 5 now permits the identification, isolation and synthesis of Hev b 5 B cell epitopes and the present invention should accordingly be understood to extend to Hev b 5 B cell epitopes and to derivatives, homologues, analogues, mutants, chemical equivalents or mimetics thereof.

In one preferred embodiment there is provided an isolated peptide of the formula:



wherein

X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;

X_2 is an amino acid sequence derived from, or homologous to Hev b 5

and wherein said peptide molecule is capable of interacting with antibody from subjects having a condition characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5 or a derivative, homologue, analogue, mutant, chemical equivalent or mimetic of said peptide.

Preferably, said condition is latex hypersensitivity and said antibodies are of the IgE isotype.

More particularly the present invention provides an isolated peptide of the formula:



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wherein

X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;

X_2 is any amino acid sequence derived from, or homologous to Hev b 5

and wherein said peptide molecule is capable of interacting with antibodies IC10, 6F6, 3G3 or 6A10 or a derivative, homologue, analogue, mutant, chemical equivalent or mimetic of said peptide.

Reference to "Hev b 5", and "peptide" should be understood to have the same meaning as hereinbefore defined. Similarly, reference to a condition involving an "aberrant, unwanted or otherwise inappropriate" immune response and "latex hypersensitivity" should also be understood to have the same meaning as hereinbefore provided. Further, reference to "derivatives, homologues, analogues, chemical equivalents and mimetics of the subject peptide or antibody also has the same meaning as provided earlier. The peptides encompassed by this aspect of the present invention may also undergo modification as hereinbefore detailed. Such modification is particularly useful for generating mutant peptides which are useful for a prophylactic and/or therapeutic treatment of individuals suffering from or predisposed to a condition characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5. For example, it may be particularly useful to generate a mutant peptide comprising T cell epitopic regions but which peptides lack B cell epitopes capable of interacting with IgE. Such peptides may be generated by synthesising peptides comprising only T cell epitopes or by mutating naturally occurring molecules such that the T cell epitopes remain functional while the B cell epitopes are altered to prevent antibody binding. It should also be understood that the subject "amino acid sequence" may correspond to the sequence of either a continuous or discontinuous epitope of Hev b 5.

The present invention should therefore be understood to encompass peptides that comprise at least one B or T cell epitope of Hev b 5 in conjunction with other amino acids (which may or may not be naturally occurring as amino acid analogues) or other chemical species.

In a preferred aspect of the invention such peptides may comprise one or more epitopes of Hev b 5, which epitopes may be T or B cell epitopes. Peptides with one or more epitopes of Hev b 5 are desirable for increased therapeutic effectiveness.

In another aspect, the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding the peptides as hereinbefore defined or a derivative, homologue or analogue thereof. It should be understood that reference to "peptides" includes reference to peptides comprising one or more T cell epitopes, one or more B cell epitopes or a combination of B and T cell epitopes. A nucleic acid molecule encoding the subject peptide is preferably a sequence of deoxyribonucleic acids such as cDNA or a genomic sequence. A genomic sequence may comprise exons and introns. A genomic sequence may also include a promoter region or other regulatory regions.

The nucleic acid molecule may be ligated to an expression vector capable of expression in a prokaryotic cell (eg. *E. coli*) or a eukaryotic cell (eg. yeast cells, fungal cells, insect cells, mammalian cells or plant cells). The nucleic acid molecule may be ligated or fused or otherwise associated with a nucleic acid molecule encoding another entity such as, for example, a signal peptide. It may also comprise additional nucleotide sequence information fused, linked or otherwise associated with it either at the 3' or 5' terminal portions or at both the 3' and 5' terminal portions. The nucleic acid molecule may also be part of a vector, such as an expression vector. The latter embodiment facilitates production of recombinant forms of the subject peptide which forms are encompassed by the present invention.

Such nucleic acids may be useful for recombinant production of T cell epitopes of Hev b 5 or proteins comprising them by insertion into an appropriate vector and transfection into a

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suitable cell line. Such expression vectors and host cell lines also form an aspect of the invention.

In producing peptides by recombinant techniques, host cells transformed with a nucleic acid having a sequence encoding a peptide according to the invention or a functional equivalent of the nucleic acid sequence are cultured in a medium suitable for the particular cells concerned. Peptides can then be purified from cell culture medium, the host cells or both using techniques well known in the art such as ion exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis or immunopurification with antibodies specific for the peptide.

Nucleic acids encoding Hev b 5 or peptides comprising T and/or B cell epitopes of Hev b 5 may be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells such as Chinese hamster ovary cells (CHO). Suitable expression vectors, promoters, enhancers and other expression control elements are referred to in Sambruck *et al* (1989). Other suitable expression vectors, promoters, enhancers and other expression elements are well known to those skilled in the art. Examples of suitable expression vectors in yeast include Yep Sec 1 (Balderi *et al.*, 1987); pMFa (Kurjan and Herskowitz, 1982); JRY88 (Schultz *et al.*, 1987) and pYES2 (Invitrogen Corporation, San Diego, CA). These vectors are freely available as are baculovirus and mammalian expression systems. For example, a baculovirus system is commercially available (ParMingen, San Diego, CA) for expression in insect cells while the pMsg vector is commercially available (Pharmacia, Piscataway, NJ) for expression in mammalian cells.

For expression in *E. coli* suitable expression vectors include among others, pTrc (Amann *et al*, 1988) pGex (Amrad Corporation, Melbourne, Australia); pMal (N.E. Biolabs, Beverley, MA); pRit5 (Pharmacia, , Piscataway, NJ); pEt-11d (Novagen, Maddison, WI) (Jameel *et al* 1990) and pSem (Knapp *et al.*, 1990). The use of pTRC, and pEt-11d, for example, will lead to the expression of unfused protein. The use of pMal, pRit5, pSem and pGex will lead to the expression of allergen fused to maltose E binding protein (pMal), protein A (pRit5), truncated -galactosidase (PSEM) or glutathione S-transferase (pGex). When a T

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cell epitope of Hev b 5 or a peptide comprising it is expressed as a fusion protein, it is particularly advantageous to introduce an enzymatic cleavage site at the fusion junction between the carrier protein and the peptide concerned. The peptide of the invention may then be recovered from the fusion protein through enzymatic cleavage at the enzymatic site and biochemical purification using conventional techniques for purification of proteins and peptides. Examples of enzymatic cleavage sites include those for blood clotting factor Xa or thrombin for which the appropriate enzymes and protocols for cleavage are commercially available. The different vectors also have different promoter regions allowing constitutive or inducible expression or temperature induction. It may additionally be appropriate to express recombinant peptides in different *E. coli* hosts that have an altered capacity to degrade recombinantly expressed proteins. Alternatively, it may be advantageous to alter the nucleic acid sequence to use codons preferentially utilised by *E. coli*, where such nucleic acid alteration would not effect the amino acid sequence of the expressed proteins.

Host cells can be transformed to express the nucleic acids of the invention using conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection or electroporation. Suitable methods for transforming the host cells may be found in Sambruck *et al* (1989), and other laboratory texts. The nucleic acid sequence of the invention may also be chemically synthesised using standard techniques.

In addition to recombinant production of peptides according to the invention, the nucleic acids may be utilised as probes for experimental or purification purposes.

The identification of both B and T cell epitopic regions facilitates the identification and/or rational design of a range of mutant peptide molecules. As detailed hereinbefore, these mutant peptides may comprise one or more mutated T cell epitopes. However, in accordance with the antibody/B cell epitope related aspect of the present invention, there is provided scope for the generation of mutant peptides comprising mutated B cell epitopes or combinations of intact versus mutated B and T cell epitopes. The applications of these

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molecules are described in more detail below but in a preferred embodiment relate to modulation of the Hev b 5 hypersensitivity immune response in terms of either a prophylactic or therapeutic treatment.

Identification and synthesis of the Hev b 5 T and B cell epitopes and generation of Hev b 5 antibodies as disclosed herein now facilitates the development of a range of diagnostic and prophylactic/therapeutic treatment protocols for use with respect to Hev b 5 related immune conditions. Also facilitated is the development of reagents for use therein. Accordingly, the present invention should be understood to extend to the use of the peptides and monoclonal antibodies or derivatives, homologues, analogues, mutants, chemical equivalents or mimetics thereof of the present invention in the therapeutic and/or prophylactic treatment of patients. Such methods of treatment include, but are not limited to:

- (i) Administration of the subject peptides to a patient as a means of desensitising or inducing immunological tolerance to Hev b 5 or Hev b 5-like molecules. This may be achieved, for example, by inducing Hev b 5 directed Th2 anergy or apoptosis. Such an outcome may be achieved by any one of a number of techniques including the use of peptides which maintain T cell epitope reactivity but which either naturally or as a result of mutation are unable to undergo IgE binding. Alternatively, one may utilise desensitisation/treatment protocols which are based on the administration of specific concentrations of a given peptide in accordance with a specific regime in order to induce high or low dose tolerance. Such methodology may eliminate Hev b 5 hypersensitivity or it may reduce the severity of Hev b 5 hypersensitivity.

Preferably such treatment regimes are capable of modifying the T cell response or both the B and T cell response of the individual concerned. As used herein, modification of the allergic response of the individual suffering from Hev b 5 hypersensitivity can be defined as inducing either non-responsiveness or diminution in symptoms to the Hev b 5 molecule as determined by standard clinical procedures

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(Varney *et al.*, 1990). Diminution in the symptoms includes any reduction in an allergic response in an individual to Hev b 5 after a treatment regime has been completed. This diminution may be subjective or clinically determined, for example by using standard skin tests known in the art.

Exposure of an individual to the peptides of the present invention, which peptides comprise at least one T cell epitope, may tolerise or anergise appropriate T cell subpopulations such that they become unresponsive to Hev b 5 and do not participate in stimulating an immune response upon such exposure. Preferably the peptides according to the invention will retain immunodominant T cell epitopes but possess abrogated IgE binding.

Administration of a peptide of the invention may modify the lymphokine secretion profile as compared with exposure to naturally occurring Hev b 5 allergen. This exposure may also influence T cell subpopulations which normally participate in the allergic response to migrate away from the site or sites of normal exposure to the allergen and towards the site or sites of therapeutic administration. This redistribution of T cell subpopulations may ameliorate or reduce the ability of an individual's immune system to stimulate the usual immune response at the site of normal exposure to the allergen, resulting in diminution of the allergic symptoms.

Modification of the B cell response may be achieved, for example, via modulation of the cytokine profile produced by T cells, as detailed above. Specifically, decreasing T cell derived IL-4 and IL-13 production thereby decreasing IgE synthesis.

- (ii) The peptides of the present invention may be used in the capacity of an adsorbent to remove Hev b 5 directed antibodies and/or T cells from a biological sample or from a patient.

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- (iii) The antibodies generated in accordance with the method of the present invention following humanisation or modification to produce Fabs, for example, may be used to abrogate or decrease Hev b 5 hypersensitivity via the administration to an individual of antibodies which bind Hev b 5 thereby competitively inhibiting binding of Hev b 5 to IgE coated mast cells, which latter binding would lead to mast cell degranulation and the onset of hypersensitivity symptoms. Although such a mechanism is unlikely to lead to long term desensitisation, it nevertheless provides a mechanism for preventing or reducing acute hypersensitivity symptom severity. This is likely to be of particular use to individuals who suffer from severe symptoms following certain types of exposure to Hev b 5, such as airway constriction.

Accordingly, in another aspect the present invention provides a method for the treatment and/or prophylaxis of a condition in a subject, which condition is characterised by the aberrant, unwanted or otherwise inappropriate immune response to Hev b 5, said method comprising administering to said subject an effective amount of a peptide and/or antibody as hereinbefore defined for a time and under conditions sufficient to remove or reduce the presence or function in said subject of T cells and/or antibodies directed to said Hev b 5.

Preferably said condition is latex hypersensitivity.

An "effective amount" means an amount necessary at least partly to attain the desired immune response, or to delay the onset or inhibit progression or halt altogether, the onset or progression of a particular condition being treated. The amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated, the degree of protection desired, the formulation of the composition, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

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The subject of the treatment or prophylaxis is generally a mammal such as but not limited to human, primate, livestock animal (e.g. sheep, cow, horse, donkey, pig), companion animal (e.g. dog, cat), laboratory test animal (e.g. mouse, rabbit, rat, guinea pig, hamster), captive wild animal (e.g. fox, deer). Preferably the mammal is a human or primate. Most preferably the mammal is a human. Although the present invention is exemplified using a murine model, this is not intended as a limitation on the application of the present invention to other species, in particular, human.

Reference herein to "treatment" and "prophylaxis" is to be considered in its broadest context. The term "treatment" does not necessarily imply that a subject is treated until total recovery. Similarly, "prophylaxis" does not necessarily mean that the subject will not eventually contract a disease condition. Accordingly, treatment and prophylaxis include amelioration of the symptoms of a particular condition or preventing or otherwise reducing the risk of developing a particular condition. The term "prophylaxis" may be considered as reducing the severity or onset of a particular condition. "Treatment" may also reduce the severity of an existing condition.

Administration of the peptide and/or antibody of the present invention (herein referred to as "agent") in the form of a pharmaceutical composition, may be performed by any convenient means. The agent of the pharmaceutical composition is contemplated to exhibit therapeutic activity when administered in an amount which depends on the particular case. The variation depends, for example, on the human or animal and the agent chosen. A broad range of doses may be applicable. Considering a patient, for example, from about 0.1 mg to about 1 mg of an agent may be administered per kilogram of body weight per day. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, weekly, monthly or other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of the situation.

The agent may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intraperitoneal, intramuscular, subcutaneous, intradermal,

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intranasal, sublingual or suppository routes or implanting (e.g. using slow release molecules). The agent may be administered in the form of pharmaceutically acceptable nontoxic salts, such as acid addition salts or metal complexes, e.g. with zinc, iron or the like (which are considered as salts for purposes of this application). Illustrative of such acid addition salts are hydrochloride, hydrobromide, sulphate, phosphate, maleate, acetate, citrate, benzoate, succinate, malate, ascorbate, tartrate and the like. If the active ingredient is to be administered in tablet form, the tablet may contain a binder such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid; and a lubricant, such as magnesium stearate.

In accordance with these methods, the agent defined in accordance with the present invention may be coadministered with one or more other compounds or molecules. By "coadministered" is meant simultaneous administration in the same formulation or in two different formulations via the same or different routes or sequential administration by the same or different routes. By "sequential" administration is meant a time difference of from seconds, minutes, hours or days between the administration of the two types of molecules. These molecules may be administered in any order.

Another aspect of the present invention contemplates the use of an agent as hereinbefore defined in the manufacture of a medicament for the treatment of a condition in a mammal, which condition is characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5.

Preferably said condition is latex hypersensitivity.

In yet another further aspect, the present invention contemplates a pharmaceutical composition comprising an agent as hereinbefore defined and one or more pharmaceutically acceptable carriers and/or diluents. Said agents are referred to as the active ingredients.

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The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion or may be in the form of a cream or other form suitable for topical application. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilisation. Generally, dispersions are prepared by incorporating the various sterilised active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be

incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μ g and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter: a binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule encoding a modulatory agent. The vector may, for example, be a viral vector.

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Yet another aspect of the present invention relates to agents, as hereinbefore defined, when used in the method of the present invention.

In yet another aspect, the present invention should be understood to extend to the use of the peptides and/or antibodies of the present invention in diagnostic applications. Said diagnostic applications include, but are not limited to:

- (i) To measure the reactivity of a subject's cells to Hev b 5. This is of use, for example, with respect to the diagnosis and/or monitoring of conditions characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5. The peptides may be added into solution or bound to a solid support together with cells derived from peripheral blood or from tissue biopsies either unfractionated, fractionated or derived as a continuous cell line. Reactivity to the subject peptide may then be measured by standard proliferation assays such as incorporation of H^3 -thymidine, standard cytotoxic assays such as release of marker radioactivity from target cells, measurement of expressed or secreted molecules such as surface markers, cytokines or other standard assays of cellular activity which are well known in the art.
- (ii) The use of T cell epitope comprising peptides together with a T cell proliferation assay which utilises a T cell sample derived from the subject will facilitate, for example, the identification of a T cell responsive population.

B cell epitope comprising peptides can be utilised to screen for the presence of antibody at the qualitative and/or quantitative levels.

- (iii) The antibodies generated in accordance with the present invention may be utilised as a diagnostic tool for screening for the presence of molecules comprising Hev b 5 cell epitopes in a sample. Said sample may be a biological sample, such as where it is suspected that an individual may have ingested Hev b 5 comprising material. For example, detection of Hev b 5 homologues in patients suffering from suspected

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fruit hypersensitivity. In another example, it may be desirable to test non-biological samples, such as those suspected of inducing hypersensitivity responses in some individuals who contact these samples, for the presence of Hev b 5. For example, it may be desirable to test rubber gloves in order to assess their qualitative or quantitative Hev b 5 composition.

The methods which can be utilised to screen for Hev b 5 on the basis of B cell epitope detection using antibody molecules are well known to those skilled in the art and include radio-allergosorbent test (RAST), paper radio immunoabsorbent test (PRIST), enzyme linked immunoabsorbent assay (ELISA), radio-immunoassay (RIA), immunoradiometric assay (IRMA), luminescence immunoassay (LIA), histamine release assays and IgE immunoblots.

As detailed above, techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays, ELISA and flow cytometry. It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of Hev b 5.

To the extent that antibody based methods of diagnosis are used, the presence of Hev b 5 may be determined in a number of ways such as by Western blotting, ELISA or flow cytometry procedures. These, of course, include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich

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assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent.

In the typical forward sandwich assay, a first antibody having specificity for the Hev b 5 or antigenic parts thereof, is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes) and under suitable conditions (e.g. 25°C) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody

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specific for a portion of the hapten. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

An alternative method involves immobilizing the target molecules in the biological sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed

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away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. "Reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic color visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

- (iv) Anti-Hev b 5 antibodies may also be utilised as a means of purifying Hev b 5 or the T or B cell epitope comprising peptides thereof which have been made by recombinant means or derived from any other source, such as natural products. Such purification techniques may be applied to isolating Hev b 5 proteins from culture supernatant fluid or from natural products, for example.

Methods of detecting Hev b 5 may be utilised, for example, to qualitatively or quantitatively detect Hev b 5 levels. However, these methods may also be utilised to screen for mutations or polymorphisms in Hev b 5 which mutations may result in, for

example, loss of T cell and/or B cell reactivity to Hev b 5. These methods may be utilised for the purpose of screening for peptide molecules suitable for use in therapeutically or prophylactically treating an individual suffering from Hev b 5 related hypersensitivity.

Accordingly, yet another aspect of the present invention is directed to a method of diagnosing or monitoring a condition in a mammal, which condition is characterised by an aberrant, unwanted or inappropriate response to Hev b 5, said method comprising screening for Hev b 5 reactive T cells and/or antibodies utilising the peptides hereinbefore defined.

Preferably said condition is latex hypersensitivity.

Still another aspect of the present invention is directed to a method of qualitatively and/or quantitatively detecting Hev b 5, or peptides thereof, in a sample said method comprising screening for said Hev b 5 or peptides thereof utilising an antibody hereinbefore defined.

In another embodiment the present invention provides diagnostic kits for use in the diagnostic methodology hereinbefore defined.

The present invention will now be further described with reference to the following non-limiting Examples.

EXAMPLE 1**Identification of human T cell epitopes of Hev b 5****(i) Methods***Subjects*

Latex allergic HCW were recruited from the Alfred Hospital Allergy Clinic (with informed consent following approval by Alfred Hospital Ethics Committee). All subjects had severe clinical symptoms of IgE mediated latex hypersensitivity and a grade 3-4/4 score of latex-specific serum IgE (Table 3) measured using the Kallestad Allercoat™ EAST system (Sanofi-Pasteur Diagnostics, USA). The latex EAST score in non-allergic individuals is 0/4 and <0.18 Allercoat™ EAST Units (AEU/ml).

Antigens

Low-ammoniated latex: Low ammoniated latex (LAL; Ansell, Australia) was centrifuged at 20,000 rpm for 20 minutes at room temperature. The middle clear layer was collected, dialysed against phosphate buffered saline (PBS) overnight at 4°C, filter sterilised and the protein concentration determined using the BCA protein assay kit (Pierce, USA).

Hev b 5: A construct comprising the Hev b 5 encoding cDNA in the pMAL/c-2 vector was generated as part of a maltose binding protein (MBP) fusion protein (rHev b 5/MBP) as described previously (Slater *et al.*, 1996). The rHEV b 5/MBP was purified from the crude bacterial protein extract by affinity chromatography on an amylose column (New England Biolabs, USA) according to the manufacturer's instructions. As a control protein, MBP fused to the LacZα protein was produced by expressing the pMAL/c-2 vector alone as described previously (Slater *et al.*, 1996).

Tetanus toxoid (TT) and Phytohaemagglutinin (PHA): These control antigens were purchased from Sigma, USA.

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Peptides: Synthetic peptides (20-mers overlapping by 11 or, for the two N-terminal peptides, 15 residues) spanning the entire length of the Hev b 5 molecule were purchased from Chiron Technologies (Clayton, Australia; Fig 1). The purity of the peptides, as determined by high-performance liquid chromatography, was greater than 90%. Peptides were dissolved in sterile PBS (Sigma, USA) to achieve a stock concentration of 1 mg/ml.

Immunoblotting

Patient serum IgE reactivity to latex allergens was analysed by dot immunoblots. LAL (25 µg), rHev b 5/MBP (7 µg) and MBP (4.5 µg) were applied side by side on a nitrocellulose membrane (Schleicher and Schuell, Germany) in a 5 µl volume and allowed to soak in. The amount of MBP was estimated to be equivalent to that in the rHev b 5/MBP aliquot. The membrane was then blocked, incubated in patient sera and IgE binding detected according to our established protocols (Suphioglu *et al.*, 1993). Briefly, the blots were blocked in 10% w/v skim milk powder in PBS and washed once in PBS containing 0.2% v/v Tween 20 and twice in PBS alone. Blots were then incubated in patients' sera (diluted 1:4 in PBS/0.5% BSA) overnight at room temperature with shaking before washing as described above. Finally, IgE antibody binding was detected by incubation in rabbit anti-human IgE (Dako, Denmark) followed by horse radish peroxidase-labelled anti-rabbit antibodies (Promega, USA), washing in between, and developing in the substrate 4-chloro-1-naphthol (Sigma) after the final wash.

Generation of latex specific T cell lines

Latex specific T cell lines were isolated using our well established methods for the generation of allergen specific oligoclonal T cell populations (O'Hehir *et al.*, 1987). An initial primary culture was performed for each patient to determine the optimal concentration of LAL for stimulation. For this, peripheral blood mononuclear cells (PBMC) separated from heparinised venous blood by density centrifugation (2×10^5 /well) were stimulated with LAL over a concentration range 0.03 to 100 µg/ml, with control cultures of PBMC alone or stimulated with tetanus toxoid. All cultures were maintained in

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complete medium (RPMI-1640, Gibco Life Technologies supplemented with 2 mmol/L L-glutamine, 100 IU/ml penicillin-streptomycin and 5% screened, heat inactivated human AB serum [Sigma]). Optimal responses were observed at 10 and 30 µg/ml LAL in all cases. Therefore, to generate T cell lines, PBMC (2.5×10^6 /well) were stimulated for 1 week with LAL at a concentration of 20 µg/ml in 24-well tissue culture plates (Costar, USA). After 7 days, lymphoblasts (1×10^6 /well) were restimulated for one week with LAL at a concentration of 20 µg/ml in the presence of an equal number of irradiated (3000 rad) autologous PBMC as antigen presenting cells (APC). On days 2 and 4, cultures were supplemented with Lymphocult-T (5% v/v; Biotest Folex, Germany) and fresh medium. At the end of the second week, lymphoblasts were restimulated for one week with rHev b 5/MBP at a concentration of 20 µg/ml as described above. Oligoclonal T cells were recovered, washed and tested in proliferation and cytokine assays (as described below) either fresh or after cryopreservation in liquid nitrogen. We have previously shown that CD4⁺ T cells are preferentially expanded in these cultures.

Oligoclonal T cell proliferation assays

Oligoclonal T cell blasts (5×10^4) were incubated in 96-well round bottom plates (Linbro, ICN Biomedicals, USA) in triplicate with equal numbers of irradiated APC in the presence of LAL at concentrations ranging from 0.03-100 µg/ml, rHev b 5/MBP (10 and 20 µg/ml), MBP (10 and 20 µg/ml), overlapping peptides of the Hev b 5 sequence (10 and 30 µg/ml), Lymphocult-T (10 IU/ml) or PHA (3 µg/ml). Cultures of T cells and APC in the absence of antigen, T cells alone and APC alone in the absence of antigen were included as controls. After 72 hours cultures were pulsed with 1 µCi of ³H-thymidine (³HTdR; Du Pont, NEN) and harvested 12-16 hours later. Proliferation as correlated with ³HTdR incorporation was measured by liquid scintillation spectroscopy. Results are expressed as mean counts per minute (cpm) for triplicate cultures with standard deviation (SD; ± 20% for all experiments).

Mitogenicity and toxicity assays

Mitogenicity of latex antigens was excluded by testing the ability of LAL, rHev b 5/MBP and Hev b 5 peptides to stimulate a house dust mite specific three-week T cell line. No reactivity to latex allergens was observed (data not shown). Toxicity was assessed by coculturing the same antigens with house dust mite specific T cells in the presence of Lymphocult-T at 10 IU/ml. Latex antigens failed to inhibit the Lymphocult-T induced proliferation even at the highest concentration (data not shown) excluding toxicity.

Production of IL-5 and IFN γ by latex specific oligoclonal T cells

The secretion of IL-5 and IFN γ by oligoclonal latex specific T cells was determined by ELISA on culture supernatants. Representative stimulatory and non-stimulatory peptides were selected and T cells cultured in triplicate with these peptides. At 48 hours 50 μ l of supernatant was harvested from the cultures and IL-5 and IFN γ were assayed by ELISA using paired cytokine antibodies (from Pharmingen Becton Dickinson, USA and Endogen, USA respectively). For this, opaque flat bottom ELISA plates (Nalgene, Nunc International) were coated with 30 μ l capture antibody at 2 μ g/ml in 0.1 M NaHCO₃ pH 8.2 coating buffer overnight at 4°C. After washing three times with PBS/0.5% Tween the wells were blocked with 3% BSA/PBS at room temperature for 2 hours. The plates were washed and 30 μ l pooled triplicate culture supernatants added in duplicate. After incubation overnight at 4°C and washing, bound cytokine was revealed by incubation with 30 μ l biotinylated detecting antibody (1 μ g/ml for IL-5, 0.5 μ g/ml for IFN γ) for 45 minutes at room temperature followed by streptavidin-biotinylated HRP (Amersham Pharmacia Biotech) at 1:2000 dilution for 30 minutes and enhanced chemiluminescence substrate (NEN Life Science). Light emission was read immediately on a Lumicount microplate Glow Luminometer (Packard Instrument Company). A standard curve was run each time using known concentrations of standard cytokine samples and the concentrations of IL-5 and IFN γ in the culture supernatants calculated accordingly. The lower limit of detection of the IL-5 assay was 20 pg/ml and the lower limit of detection of the IFN- γ assay was 2pg/ml.

(ii) Results*Serum IgE response to Hev b 5*

Sera from the latex allergic HCW were tested for IgE reactivity to latex allergens by dot immunoblotting using LAL and rHev b 5/MBP as antigens. MBP was included as a control to exclude the possibility that binding of patient sera to the fusion protein was due to anti-MBP antibodies. All patients except patient 2 demonstrated IgE reactivity to LAL (Fig 2). Sera from patients 1, 2, 3, 4 and 5 showed IgE reactivity to rHev b 5/MBP which was markedly stronger than that to MBP alone indicating the presence of a B cell response to Hev b 5 in these patients. Patient 6 showed IgE reactivity to LAL but not to the rHev b 5/MBP fusion protein indicating that Hev b 5 was not a significant allergen in this case.

T cell responses to latex allergens

In preliminary primary cultures of PBMC, the optimal response to LAL was observed at 10 or 30 $\mu\text{g/ml}$ in all cases. A representative polyclonal response is shown in Fig 3, (A). The polyclonal responses to rHev b 5/MBP were considerably less than those to LAL. A similar response was observed to MBP alone indicating the presence of MBP-specific T cells in the peripheral blood. Therefore, to enrich for latex specific T cells while preventing the selective expansion of MBP-specific T cells, LAL at 20 $\mu\text{g/ml}$ was used for primary and secondary stimulations. Finally, Hev b 5 specific T cells were enriched by incubating the LAL specific two-week T cell line with rHev b 5/MBP at 20 $\mu\text{g/ml}$ in the third antigen stimulation. The three-week latex specific T cell lines from all six latex allergic donors responded to LAL (Table 4). The T cell lines from patients 1 and 2 were also tested for their proliferative responses to rHev b 5/MBP and MBP alone. In contrast to the polyclonal responses, the oligoclonal responses to rHev b 5/MBP were significantly higher than those of MBP alone, indicating a selective expansion of Hev b 5-specific T cells. In fact, MBP responses were only at background levels. Representative responses to LAL, rHev b 5/MBP and MBP are shown Fig 3, (B).

Hev b 5 T cell epitope mapping

The latex specific three-week T cell lines were tested for proliferative responses to the Hev b 5 peptides. T cell reactivity to one or more Hev b 5 peptides was identified in all five donors who demonstrated IgE binding to rHev b 5/MBP (Table 4), and a representative T cell proliferative response to the peptide set is shown (Fig 3, B). Patient 6 failed to respond to any peptide. Hev b 5 (46-65) induced T cell proliferation in all five donors with IgE reactivity to rHev b 5/MBP. The stimulation indices observed for these responses were 5.9, 10.8, 5.0, 5.9 and 5.6. Additionally peptide Hev b 5 (109-128) stimulated T cells from three of the five donors.

Production of IL-5 and IFN γ by oligoclonal latex specific T cells

IL-5 was produced by the latex specific T cell line from patient 4 in response to stimulation with selected Hev b 5 peptides that caused T cell proliferation (Fig. 4). Peptides Hev b 5 (37-56), (45-65), (55-74), (64-83) and (109-128) induced T cell proliferation (stimulation indices greater than or equal to 2.5) and also secretion of IL-5 while peptide Hev b (100-119) failed to stimulate either T cell proliferation or cytokine secretion. In contrast secretion of IFN γ by T cells that proliferated in response to Hev b 5 peptides was minimal in all cases (≤ 100 pg/ml). See Table 5.

EXAMPLE 2**Generation & Characterisation of Hev b 5 specific monoclonal antibodies****(i) Materials and Methods***Antigens*

rHev b 5/MBP - The cDNA encoding Hev b 5 was expressed as a fusion protein with MBP in the pMAL/c-2 vector as described previously (Slater *et al.* 1996). The rHev b 5/MBP was purified by affinity chromatography on an amylose column (New England Biolabs,

USA) according to the manufacturer's instructions. MBP encoding DNA fused to the LacZ α protein was produced by expressing the pMAL/c-2 vector alone and was used as the negative control protein. The rHev b 5/MBP fusion protein digest was made by incubating 500 μ g rHev b 5/MBP with 10U Factor Xa (Sigma, USA) and 0.03% SDS for 24 hours at 37°C.

Non Ammoniated Latex (NAL) - fresh sap was collected from commercial *Hevea* trees into a cooling vessel and snap frozen (Rubber Research Institute of Malaysia, Malaysia) before transport to our facility on dry ice. The raw latex was then thawed and the coagulum removed. The sap was then centrifuged for 1 hour at 50,000g and the C-serum removed for protein estimation using the Bradford assay (Biorad, USA) with bovine gamma globulin (Biorad, USA) as standard. It was then mixed 50% v/v in glycerol and stored at -20°C.

Monoclonal Antibody Production

Monoclonal antibody production was carried out based on the method of Köhler and Milstein (Köhler *et al.*, 1975) according to established protocols (Goding, 1996). Six to 8 week old female BALB/c mice were immunised intraperitoneally (IP) with 20 μ g/injection of rHev b 5/MBP diluted in 200 μ l of phosphate buffered saline (PBS). The first injection was given with an equal volume (200 μ l) of Complete Freund's Adjuvant (Sigma, USA), and the second with 200 μ l of Incomplete Freund's Adjuvant (Sigma, USA). The subsequent 4 immunisations were given in phosphate buffered saline (PBS) alone. The initial 2 immunisations were given at a 2 weekly interval; the remainder were given at monthly intervals, with 100 μ l of whole blood being taken before the first immunisation and at 5 days following each immunisation (except for the first) to monitor the immune response. The blood was then centrifuged, the serum removed, and aliquotted at -20°C for later analysis using enzyme linked immunosorbent assay (ELISA) as below. The final booster immunisation of 20 μ g of rHev b 5/MBP in 200 μ l PBS was given IP 3 days before the mouse was humanly killed and the spleen aseptically removed, macerated and then fused with 10^7 murine myeloma cells (X-63.Ag8 cell line, generous gift of Professor Jim

Goding). The fusion method used was with 50% PEG mw 1300-1600 (Sigma, USA), with gentle mixing at 37°C for 4 minutes. The fusion was then diluted into 60 mls of selection medium: RPMI 1640 (Life Technologies, USA) supplemented with 20% fetal calf serum (FCS) (CSL, Australia), 10 mls of 50xHAT medium (Sigma, USA, 5 mls of Penicillin-Streptomycin-Glutamine (PSG) (Life Technologies, USA) and 10 mls of Oxalate-Pyruvate Insulin supplement (OPI) (Sigma, USA). This cell suspension was then plated out into 6 flat bottom 96 well tissue culture plates (100 µl/well) (Linbro, USA) and incubated at 37°C with 7% CO₂. Further fresh medium (100 µl per well of RPMI with 10% FCS, HAT, PSG) was added 5 days following fusion. Seven days following fusion hybridomas were visible by eye in many wells and the first screening was performed. Supernatants were removed (100 µl per well) and transferred to microtitre trays. A duplicate ELISA was then performed for each plate against rHev b 5/MBP and MBP alone. Briefly, ELISA plates (Costar, USA) were coated overnight (O/N) with 50 µl per well of rHev b 5/MBP or MBP alone at 0.2 µg/ml in ELISA coating buffer pH 9.5 (2.52 g NaHCO₃ + 1.59 g Na₂CO₃ per 500 ml distilled H₂O). The plates were then washed 8 times in PBS with 0.5% Tween 20 (BDH, England) (PBST) and then blocked with 5% w/v skim milk powder (SMP) in PBST O/N at 4°C before being washed again 8 times in PBST. Hybridoma supernatants were then incubated (50 µl/well) for 1 hour at 37°C, the plates then washed 8 times in PBST then 50 µl per well of horse-radish peroxidase (HRP) labelled sheep anti-mouse antibody (Silenius, USA) diluted 1:5000 in 1% bovine serum albumin (BSA) PBST was added and incubated for 1 hour at 37°C. The plates were then washed 4 times with PBST and then 4 times with PBS alone before the addition of 50 µl/well of a solution of 50 mg O-phenylene diamine (OPD)(Sigma, USA) in 12.5 ml of phosphate citrate buffer with perborate (Sigma, USA). This was incubated for 10 minutes at 37°C and the reaction stopped with 4M HCl. Colour development was then observed and the plate read at 490 nm. In all cases, one well of the ELISA plate had cell culture medium alone as a negative control and the terminal bleed of the fusion mouse at a 1:5000 dilution in 1% BSA PBST as positive control.

Positive clones were then transferred to 24 well plates (Costar, USA) in RPMI with 10% FCS, PSG and HT supplement (Sigma, USA) then expanded before cloning by limiting dilution in flat bottom 96 well plates. Limiting dilution cloning was performed in the

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presence of feeder cells (splenocytes from female BALB/c mice) suspended in RPMI 1640, 10% FCS, PSG, HAT and OPI. Positive clones were again screened by ELISA against rHev b 5/MBP, MBP at a concentration of 10 µg/ml in ELISA coating buffer pH 9.5.

Western blotting

Extracts of NAL (35 µg/lane) and rHev b 5/MBP digest (10 µg/lane) were resolved on 12% SDS-PAGE (125V DC for 90 minutes) with Benchmark pre-stained protein ladder (Life Technologies, USA) as molecular weight standards. Gels were then stained with Coomassie Brilliant Blue for total protein visualisation or electroblotted to nitrocellulose (NC) membranes (Schleicher and Schuell, Germany) for 45 minutes at 25V DC. After transfer, NC membranes were then stained with 0.1% Ponceau S to ensure protein transfer and cut into strips. They were then blocked with 5% SMP in PBST for 1 hour at RT, washed twice in PBST and then incubated with hybridoma supernatants or mouse serum for 1 hour at RT. Blots were then washed 5 times in PBST and then incubated in sheep anti-mouse HRP diluted 1:10,000 in PBST. The blots were then washed 5 times in PBST and then incubated in enhanced chemiluminescence reagent (ECL)(NEN Life Sciences, USA) as per the manufacturer's instructions and luminescence detected on autoradiography film (Kodak, Australia).

(ii) Results

The mice generated a strong polyclonal immune response to rHev b 5/MBP, MBP and NA as demonstrated by ELISA (Fig. 6). The response to rHev b 5/MBP and NAL was stronger than that to MBP alone, indicating a specific immune response to rHev b 5. Five hybridomas with the desired antibody specificity (positive for rHev b 5/MBP but negative to MBP were generated). Three of these (1C10, 6F6 and 3G3) also recognised NAL strongly, the other 2 recognising NAL much less so (Fig. 7). Western blotting of rHev b 5/MBP digest showed that MoAb 1C10 detected rHev b 5/MBP and rHev b 5 but not MBP alone (Fig. 8). When blots of NAL were probed with MoAb 1C10, a protein band at 24 kDa was strongly detected, while a band at 80 kDa was also detected, though much less so (Fig. 9).

EXAMPLE 3

B cell epitope mapping of Hev b 5

It is noteworthy that, unlike some other major allergens, the amino acid sequence of Hev b 5 lacks cysteine residues, which are involved in disulphide bonds and thus stabilisation of the tertiary structure of a protein. This indicates that some of the IgE epitopes of Hev b 5 are linear or continuous, which makes the synthetic peptide approach of epitope mapping useful, as has been previously found in Lol p 5 (Suphioglu et al. 1998).

Synthetic overlapping peptides based on the deduced amino acid sequence of the mature Hev b 5 protein (fourteen 20-mers with 11 amino acid overlap) are used to identify linear B cell epitopes following established protocols (Suphioglu et al. 1998). Briefly, synthetic peptides are immobilised either on nitrocellulose membranes or microtitre plates, non-binding sites blocked with 3% BSA/PBS and IgE-binding of patients' sera detected with mouse anti-human IgE followed by horse radish peroxidase conjugated anti-mouse antibodies. Total non-ammoniated latex (NAL) and glove extract (GE) proteins and recombinant Hev b 5 (recHev b 5) are immobilised also, as positive controls. IgE reactive Hev b 5 peptides, along with NAL, GE proteins and recHev b 5, as positive controls, are evaluated for their capacity to inhibit IgE binding to the natural and recHev b 5 allergens. This indicates whether or not the identified IgE-reactive peptides correspond to IgE-reactive epitopes revealed within the context of the natural and recHev b 5 allergens.

Conformational B cell epitopes of Hev b 5 (if any) are identified with random mutagenesis. In this strategy, the plasmid vector containing the cDNA encoding IgE-reactive recombinant Hev b 5 is transformed into XL1-Red host cells (Epicurian Coli, Stratagene, CA, USA) to generate random mutations within the cDNA encoding Hev b 5. The XL1-Red strain is deficient in three of the primary DNA repair pathways in *E. coli* (ie. *mutS*, *mutD* and *mutT*) making its mutation rate approximately 5,000-fold higher than that of its wild-type parent. After transformation and propagation in XL1-Red strain, plasmid DNA is retrieved and transformed into XL1-Blue strain for analysis. This is necessary as every

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time the plasmid replicates in the XL1-Red strain, further mutations occur. Once transformed into the XL1-Blue, different dilutions of the transformants are plated to obtain single colonies for immunoscreening with IgE sera (which has been preabsorbed with *E.coli* extract) known to possess recHev b 5-specific IgE. Immunoscreening of bacterial colonies identifies mutants defective in IgE-binding, as compared to non-mutated recHev b 5 control, and responsible mutations will be resolved by DNA sequencing. Such B cell epitope sequences are compared with the linear B cell epitopes identified above to determine if conformational epitopes have been resolved.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

Table 3 - Clinical characteristics of latex allergic patients

Patient	Age	Sex	Clinical symptoms on contact with latex	Clinical food allergy	Clinical kiwi fruit allergy	Latex EAST score out of 4 (assay result in AEU/ml)
1	37	F	Urticaria, asthma, angioedema, anaphylaxis	Yes	No	4 (16.4)
2	55	F	Urticaria, angioedema	Yes	Yes	3 (7.1)
3	41	F	Urticaria, rhinitis, asthma, angioedema, anaphylaxis	Yes	Yes	3 (9.5)
4	65	F	Urticaria, rhinitis, asthma, angioedema, anaphylaxis	Yes	Yes	4 (>17.5)
5	30	F	Urticaria, angioedema	Yes	Unknown	4 (>17.5)
6	41	F	Urticaria, rhinitis, asthma, angioedema, anaphylaxis	Yes	Unknown	3 (9.5)

Table 4 Oligoclonal proliferative responses to Hev b 5 peptides of latex allergic donors

Antigen		P1	P2	P3	P4	P5	P6
T+APC	alone	3.6	2.1	5.2	0.9	1.0	1.0
LAL	100	9.5	12.6	55.9	2.2	4.1	29.9
	30	7.8	9.8	65.9	2.9	4.6	27.9
	10	8.8	9.4	63.4	3.1	3.3	14.6
	3	7.1	7.7	37.1	2.0	1.7	9.9
Hev b 5-MBP		9.0	5.7	NT	NT	NT	NT
MBP		2.5	1.5	NT	NT	NT	NT
(1-20)	30	2.5	2.1	8.2	2.0	2.6	2.2
	10	3.0	1.6	7.6	1.6	0.9	1.0
(10-29)	30	3.3	1.7	5.2	1.2	0.8	1.7
	10	3.2	1.4	7.7	1.6	1.5	1.1
(19-38)	30	2.4	2.4	3.0	1.6	4.0	2.0
	10	2.4	1.1	6.4	1.2	0.6	0.9
(28-47)	30	5.0	41.7	6.9	1.4	1.6	1.1
	10	7.2	25.8	6.4	1.2	0.8	0.9
(37-56)	30	8.5	38.8	2.7	3.2	1.1	0.9
	10	7.1	18.3	8.8	3.0	1.4	1.4
(46-65)	30	19.6	22.6	19.2	5.3	5.6	1.2
	10	21.3	13.4	25.8	4.4	4.9	1.5
(55-74)	30	7.7	5.6	8.2	2.8	0.7	0.9
	10	5.0	2.8	9.1	2.0	1.1	0.7
(64-83)	30	5.6	2.1	4.3	2.6	2.4	1.0
	10	3.5	1.8	7.0	1.9	0.5	0.8
(73-92)	30	2.9	14.6	3.1	1.2	0.5	1.3
	10	2.5	9.8	5.3	1.2	0.8	1.0
(82-101)	30	5.2	2.1	6.5	2.6	1.6	1.2
	10	4.1	3.2	7.3	2.4	1.3	0.8
(91-110)	30	2.6	3.2	8.1	2.2	3.2	1.2
	10	2.4	2.4	7.4	1.6	1.1	0.7
(100-119)	30	2.0	2.2	8.0	1.1	0.8	1.0
	10	2.4	1.3	6.8	1.2	1.0	0.7
(109-128)	30	5.6	42.7	17.3	3.0	0.9	0.6
	10	8.2	36.9	13.7	2.3	1.3	0.9
(118-137)	30	2.5	20.8	8.5	1.2	1.3	0.8
	10	3.9	19.0	6.8	0.9	0.5	0.9
(127-146)	30	4.4	6.6	8.3	1.6	2.5	0.8
	10	3.3	3.8	7.1	2.2	2.7	1.0
(132-151)	30	6.5	3.3	7.0	1.7	1.0	0.8
	10	4.1	2.7	6.7	1.1	1.4	1.2

Latex specific 3-week T cell lines from latex allergic patients (P1-P6) were stimulated with the Hev b 5 peptides at 10 and 30 µg/ml, LAL at 3, 10, 30 and 100 µg/ml and rHev b 5/MBP at 10 µg/ml in 4 day cultures. The responses for each peptide and protein antigen are given as counts per minute $\times 10^{-3}$ and compared with the control response where T cells and APC were incubated alone in the absence of antigen. Stimulation indices of ≥ 2.5 are shown in bold type. NT = not tested.

Table 5 IL-5 and IFN γ levels produced by latex specific T cell lines when stimulated with Hev b 5 peptides

Patient	Hev b5 Peptides tested (30,10 μ g/ml)	IL-5 (pg/ml)		IFN γ (pg/ml)		IL-5/IFN γ ratio	
		30	10	30	10	30	10
P1	28-47 (-)	68	0	0	0	-	-
	37-56 (-)	0	0	0	0	-	-
	46-65 (+)	279	344	2	2	139.5	172
	55-74 (-)	0	0	0	0	-	-
	64-83 (-)	0	14	0	0	-	-
	73-92 (-)	0	0	0	0	-	-
	100-109 (-)	0	0	0	0	-	-
	109-128 (-)	69	0	0	0	-	-
P2	28-47 (+)	678	706	112	122	6.1	5.8
	37-56 (+)	767	744	77	74	10	10.1
	46-65 (+)	408	58	54	18	7.6	3.2
	55-74 (-)	0	0	4	2	-	-
	64-83 (-)	0	0	4	2	-	-
	73-92 (+)	287	141	45	18	6.4	7.8
	100-119 (-)	17	0	4	2	4.2	-
	109-128 (+)	595	293	97	73	6.1	4
P3	46-65 (+)	3186	3799	227	388	14	9.8
	64-83 (-)	205	109	129	132	1.6	0.8
	73-92 (-)	24	27	83	112	0.3	0.2
	109-128 (+)	1559	1403	292	257	5.3	5.5
P4	37-56 (+)	296	173	6	0	49.3	173
	46-65 (+)	519	561	12	25	43.2	22.4
	55-74 (+)	199	70	0	0	199	70
	64-83 (+)	56	0	4	0	14	0
	82-101 (+)	280	285	0	0	280	285
	100-119 (-)	0	0	0	0	-	-
	109-128 (+)	212	166	0	0	212	166
	118-137 (-)	0	0	0	0	-	-1
P5	Not Tested						

Supernatants were removed at 48 hours from T cell cultures and tested in an ELISA for the presence of IL-5 and IFN γ . Proliferation inducing and non-inducing peptides are indicated by (+) and (-) respectively. The levels of IL-5 and IFN γ produced in pg/ml are shown at peptide concentrations of 30 and 10 μ g/ml. Where both cytokines were detectable the IL-5/IFN γ ratio is shown at peptide concentrations of 30 and 10 μ g/ml.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An isolated peptide of the formula:



wherein:

X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;

X_2 is any amino acid sequence derived from or homologues to Hev b 5.

and wherein said peptide molecule is capable of interacting with T cells and modifying T cell function when incubated with cells from subjects having a condition characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5 or a derivative, homologue, mutant, chemical equivalent or mimetic of said peptide.

2. The peptide according to claim 1 wherein X_2 is an amino acid sequence of from 5 to 100 residues derived from, homologous to or contiguous with amino acids 1-151 inclusive or derivatives thereof of Hev b 5.
3. The peptide according to claim 2 wherein said amino acid sequence is derived from, homologous to or contiguous with amino acids 37-74 or 109-146 inclusive or derivatives thereof of Hev b 5.
4. The peptide according to claim 2 wherein said amino acid sequence is derived from, homologous to or contiguous with amino acids 1-20, 19-110 and/or 109-146 inclusive or derivatives thereof of Hev b 5.

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5. The peptide according to claim 2 wherein said amino acid sequence is derived from, homologous to or contiguous with amino acids 37-56, 46-65, 55-74, 109-128 and/or 127-146 inclusive or derivatives thereof of Hev b 5.

6. The peptide according to claim 5 wherein said amino acids are 46-65 and/or 109-128 inclusive or derivatives thereof of Hev b 5.

7. The peptide according to claim 6 wherein said amino acids are 51-60 inclusive or derivatives thereof of Hev b5.

8. The peptide according to claim 2 wherein said amino acid sequence comprises at least 5 amino acids derived from one or more of the following amino acid sequences:

ASEQETADATPEKEEPTAAP <400>6

TPEKEEPTAAPAEPEAPAE <400>7

APAEPEAPAPETEKAAEEVEK <400>8

TKETETEAPAAPAEGEKPAE <400>14

AEEKPITEAAETATTEVPV <400>16.

9. The peptide according to claim 8 wherein said amino acid sequence is derived from one or more of <400>7 or <400>14.

10. The peptide according to claim 9 wherein said amino acid sequence is derived from <400>7.

11. The peptide according to claim 10 wherein said amino acid sequence is derived from the following amino acid sequence:

EPTAAPAEPE <400>42.

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12. An isolated peptide comprising an amino acid sequence derived from or homologous to Hev b 5 wherein said peptide molecule is capable of interacting with T cells and modifying T cell function when incubated with cells from subjects having a condition characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5 or a derivative, homologue, analogue, mutant, chemical equivalent or mimetic of said peptide.

13. The peptide according to claim 12 wherein said amino acid sequence is of 5-100 residues derived from, homologous to or contiguous with amino acids 1-151 inclusive or derivatives thereof of Hev b 5.

14. The peptide according to claim 13 wherein said amino acid sequence is derived from, homologous to or contiguous with amino acids 37-74 or 109-146 inclusive or derivatives thereof of Hev b 5.

15. The peptide according to claim 13 wherein said amino acid sequence is derived from, homologous to or contiguous with amino acids 1-20, 19-110 and/or 109-146 inclusive or derivatives thereof of Hev b 5.

16. The peptide according to claim 13 wherein said amino acid sequence is derived from, homologous to or contiguous with amino acids 37-56, 46-65, 55-74, 109-128 and/or 127-146 inclusive or derivatives thereof of Hev b 5.

17. The peptide according to claim 16 wherein said amino acids are 46-65 and/or 109-128 inclusive or derivatives thereof of Hev b 5.

18. The peptide according to claim 17 wherein said amino acids are 51-60 inclusive or derivatives thereof of Hev b 5.

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19. The peptide according to claim 13 wherein said amino acid sequence comprises at least 5 amino acids derived from one or more of the following amino acid sequences:

ASEQETADATPEKEEPTAAP <400>6

TPEKEEPTAAPAEPEAPE <400>7

APAEPEAPAPETEKAAEEVEK <400>8

TKETETEAPAAPAEGEKPAE <400>14

AEEKPITEAAETATTEVPV <400>16.

20. The peptide according to claim 19 wherein said amino acid sequence is derived from one or more of <400>7 or <400>14.

21. The peptide according to claim 20 wherein said amino acid sequence is derived from <400>7.

22. The peptide according to claim 21 wherein said amino acid sequence is derived from the following amino acid sequence:

EPTAAPAEPE <400>42.

23. An antibody directed to Hev b 5 or derivative, homologue, analogue, chemical equivalent or mimetic thereof.

24. The antibody according to claim 23 wherein said antibody is a polyclonal antibody.

25. The antibody according to claim 23 wherein said antibody is a monoclonal antibody.

26. The antibody according to claim 25 wherein said antibody is 1C10, 6F6, 3G3 or 6A10.

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27. An isolated peptide of the formula:



wherein:

X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 non-naturally occurring amino acid residues;

X_2 is an amino acid sequence derived from or homologous to Hev b 5

and wherein said peptide molecule is capable of interacting with antibody from subjects having a condition characterised by aberrant, unwanted or otherwise inappropriate immune response to Hev b 5 or a derivative, homologue, analogue, mutant, chemical equivalent or mimetic of said peptide.

28. The peptide according to claim 27 wherein said antibody is 1C10, 6F6, 3G3 or 6A10.

29. An isolated peptide comprising an amino acid sequence derived from or homologous to Hev b 5 wherein said peptide is capable of interacting with antibody from subjects having a condition characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5 or a derivative, homologue, analogue, mutant, chemical equivalent or mimetic of said peptide.

30. The peptide according to claim 29 wherein said antibody is 1C10, 6F6, 3G3 or 6A10.

31. An isolated nucleic acid sequence encoding or complementary to a sequence encoding the peptide according to any one of claims 1-22 or 27-30.

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32. A method for the treatment and/or prophylaxis of a condition in a subject, which condition is characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5, said method comprising administering to said subject an effective amount of a peptide according to any one of claims 1-22 or 27-30 and/or an antibody according to any one of claims 23-26 for a time and under conditions sufficient to remove or reduce the presence or function in said subject of T cells and/or antibodies directed to said Hev b 5.
33. The method according to claim 32 wherein said condition is latex hypersensitivity.
34. Use of a peptide and/or antibody according to any one of claims 1-30 in the manufacture of a medicament for the treatment of a condition in a mammal which condition is characterised by an aberrant, unwanted or otherwise inappropriate immune response to Hev b 5.
35. Use according to claim 34 wherein said condition is latex hypersensitivity.
36. A pharmaceutical composition comprising a peptide and/or antibody according to any one of claims 1-30 together with one or more pharmaceutically acceptable carriers and/or diluents.
37. A method of diagnosing or monitoring a condition in a mammal, which condition is characterised by an aberrant, unwanted or inappropriate response to Hev b 5, said method comprising screening for Hev b 5 reactive T cells and/or antibodies utilising the peptides according to any one of claims 1-22 or 27-30.
38. The method according to claim 37 wherein said condition is latex hypersensitivity.
39. A method of qualitatively and/or quantitatively detecting Hev b 5, or peptides thereof, in a sample said method comprising screening for said Hev b 5 or peptides thereof utilising an antibody according to any one of claims 23-26.

- 73 -

40. A diagnostic kit for use in the method of any one of claims 37-39 wherein said kit comprises a peptide and/or antibody according to any one of claims 1-30.

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p(91-110)	EEKPAEPEPVAEEEEPKHETK	
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p(118-137)	AAPAEGEKPAEEEEKPITEAA	
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p(132-151)	PITEAAETATTEVPVEKTEE	

Figure 1

2/14

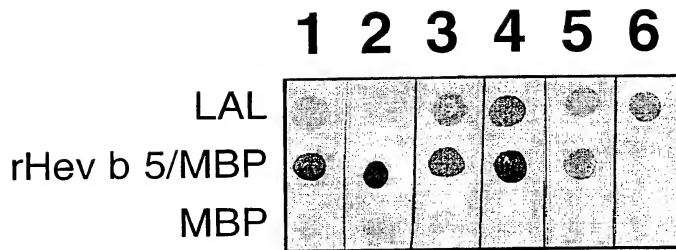


Figure 2

3/14

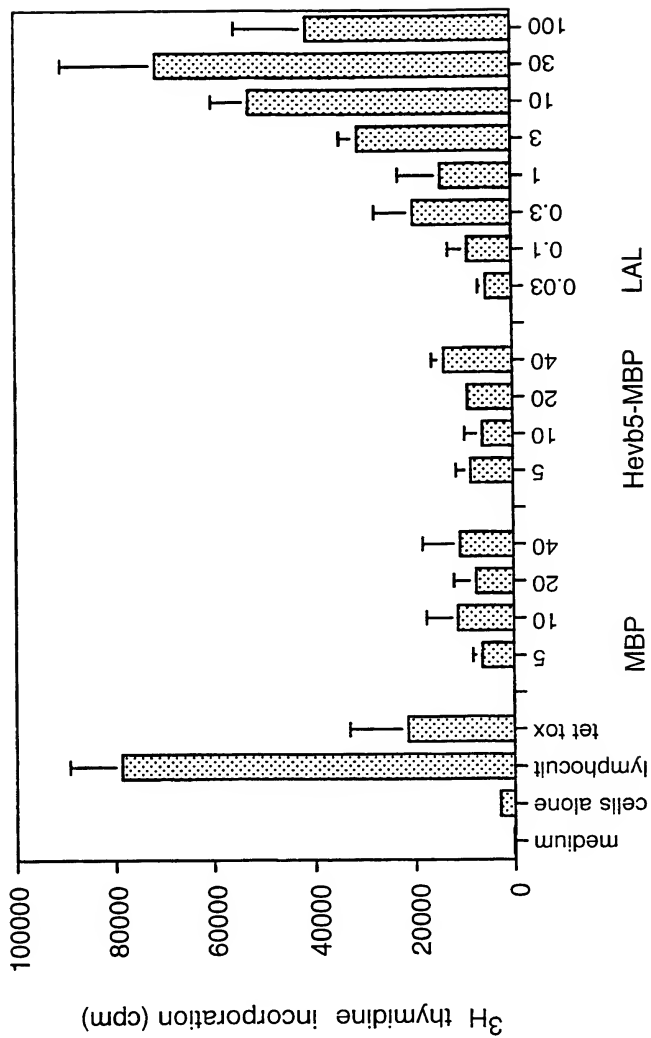
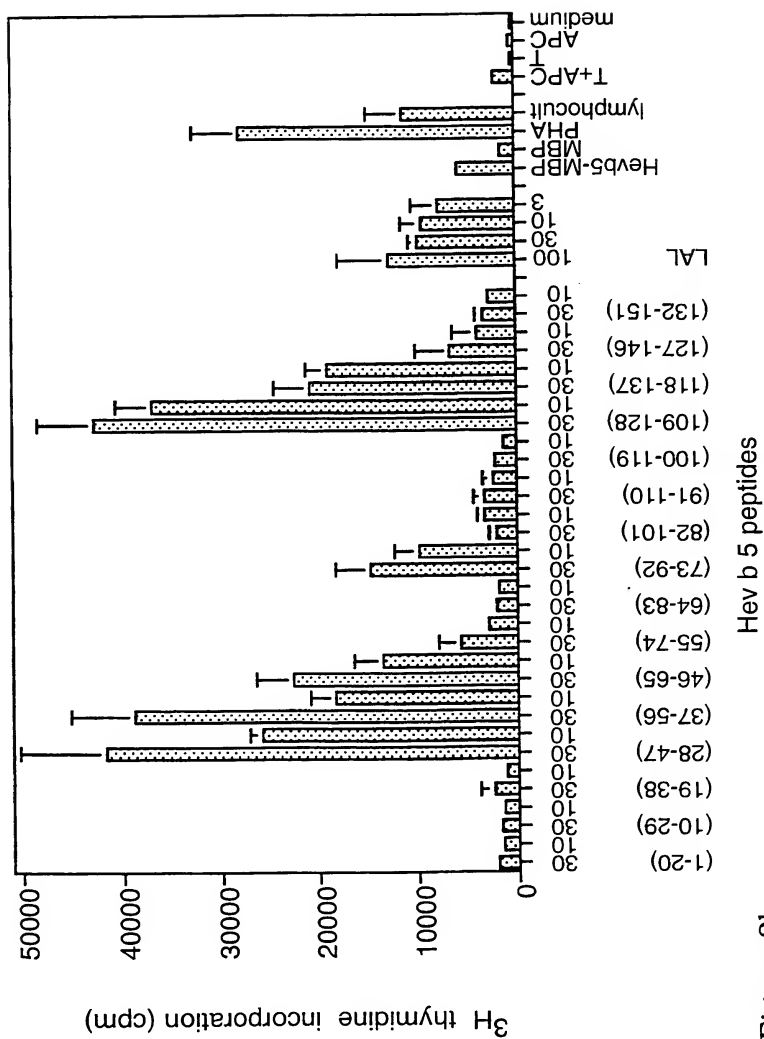


Figure 3a

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5/14

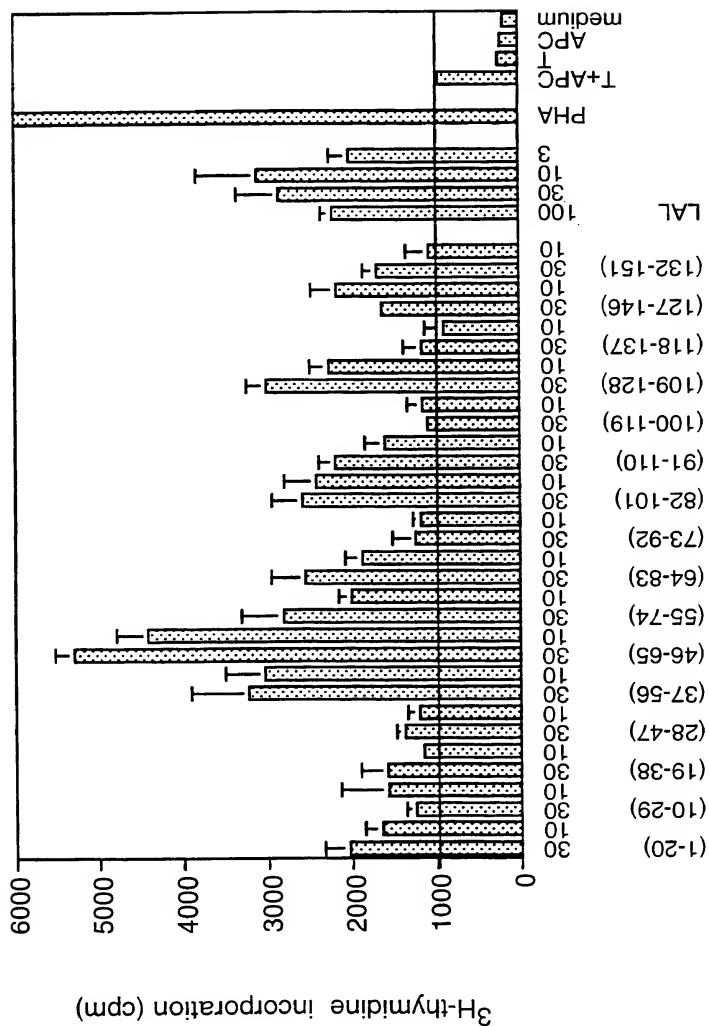


Figure 4a

6/14

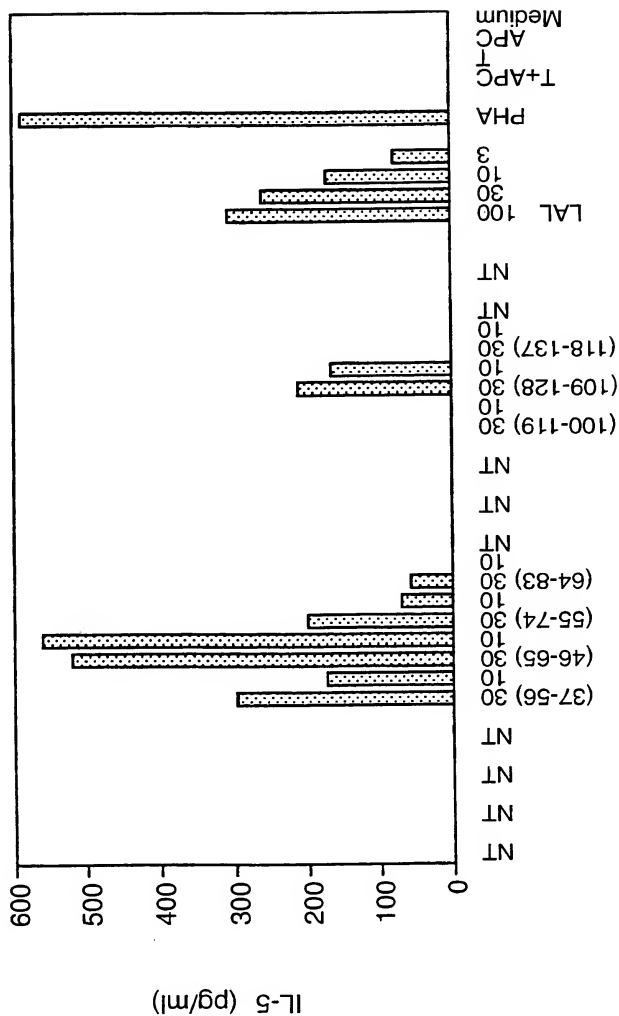


Figure 4b

7/14






























Hev b 5 peptide	Human					Murine
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10-29						
19-38						
28-47						
37-56						
46-65						
55-74						
64-83						
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91-110						
100-119						
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118-137						
127-146						
132-151						

Figure 5

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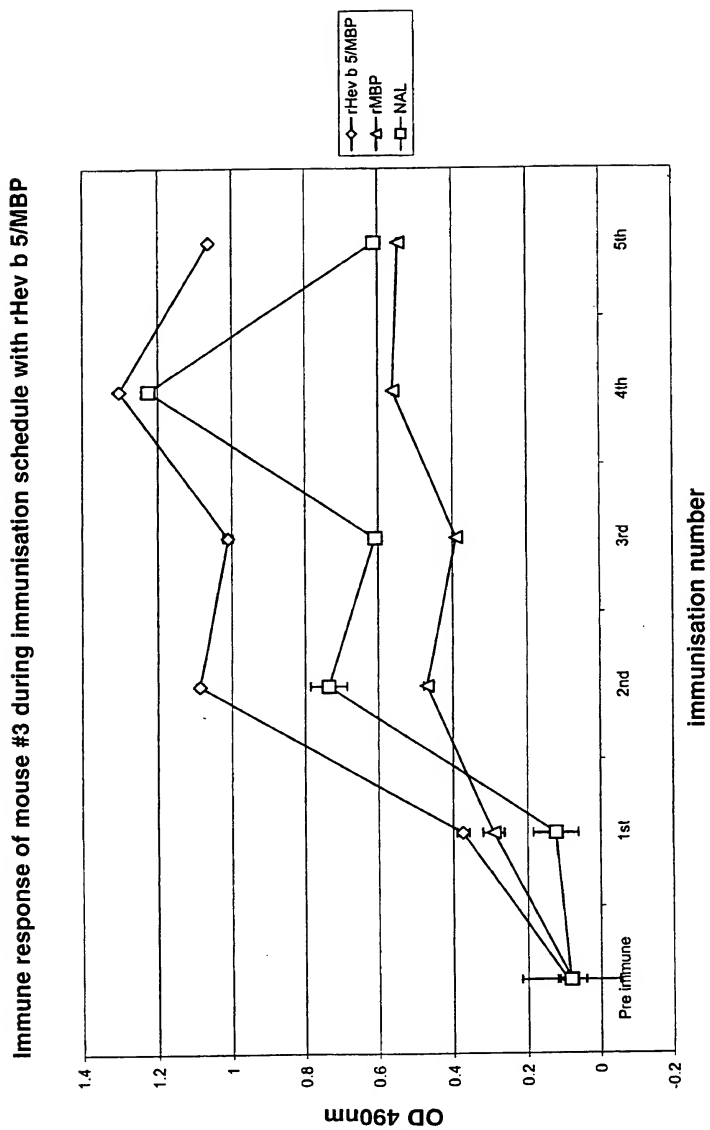


Figure 6

9/14

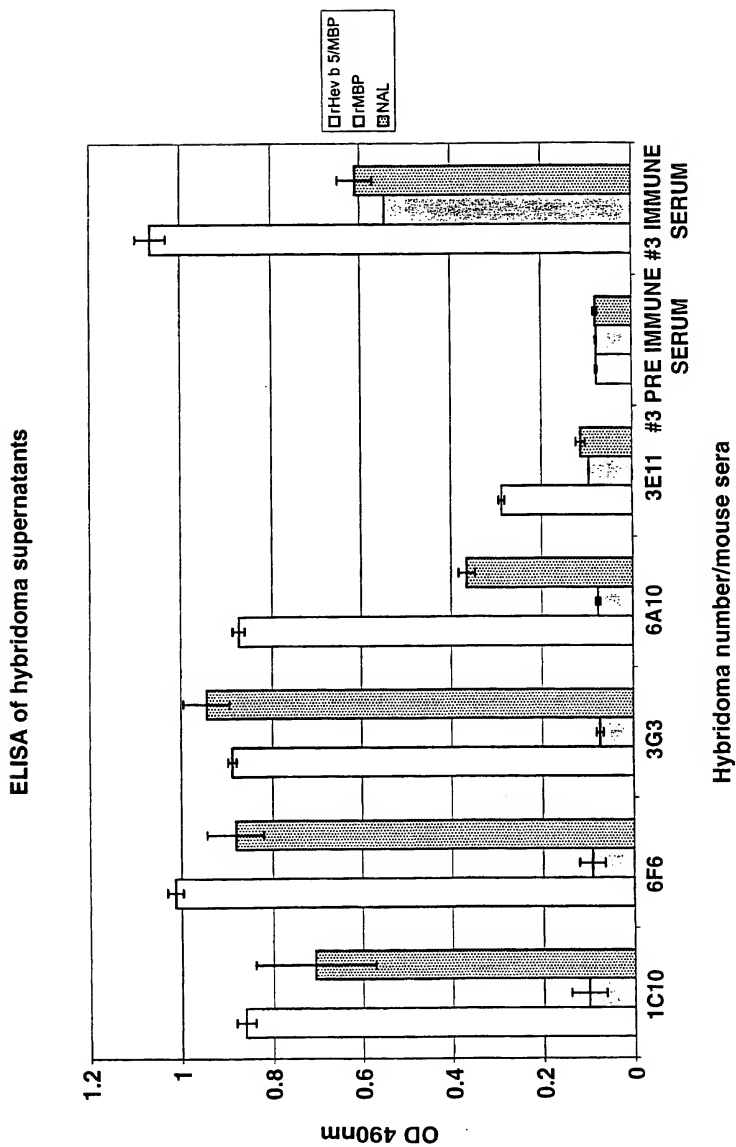
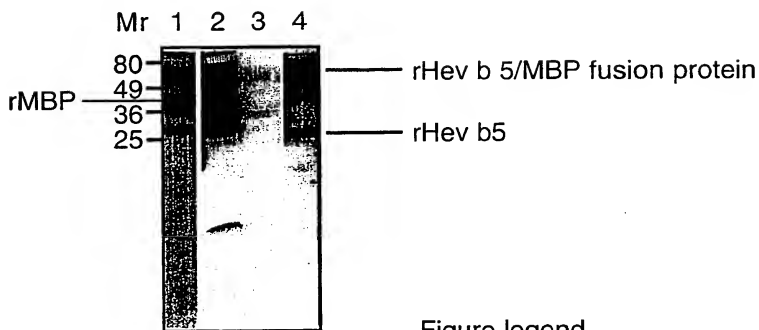


Figure 7

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Western blot of rHev b 5 fusion protein digest**Figure legend**

1. Coomassie stain
2. Polyclonal immune serum
3. Pre-immune serum
4. MoAb 1C10

Figure 8

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Western blot of NAL

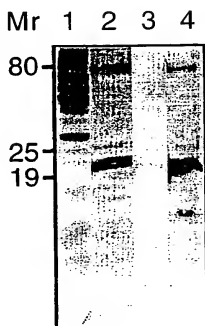
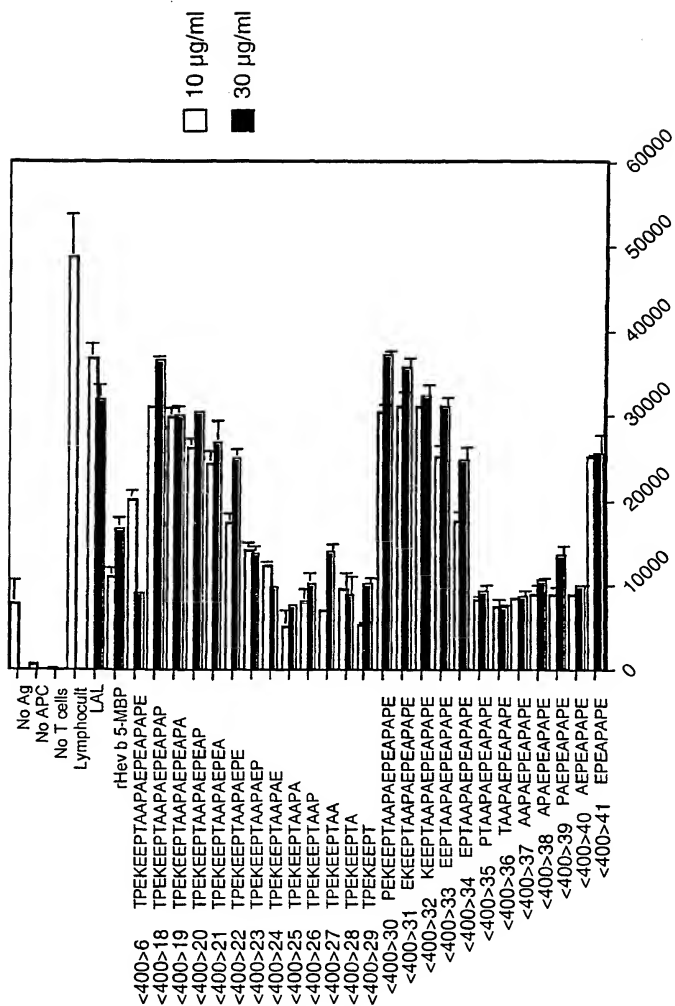


Figure legend

1. Coomassie stain
2. Polyclonal immune serum
3. Pre-immune serum
4. MoAb 1C10

Figure 9

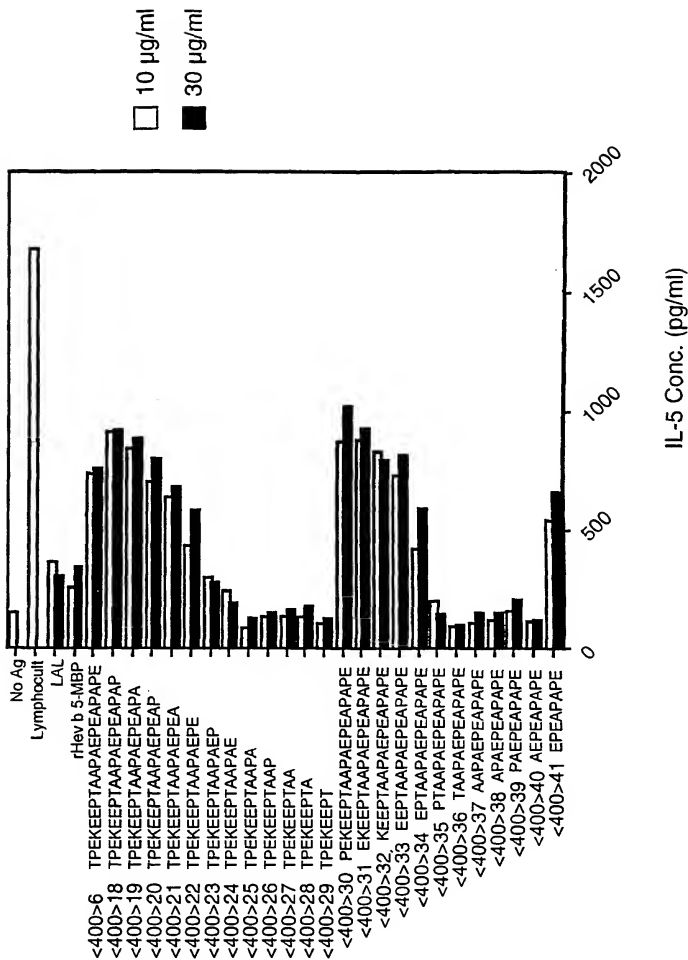
12/14



³H-Thymidine Incorporation (CPM)
Minimal epitope: EPTAAPAEPE <400>42

Figure 10a

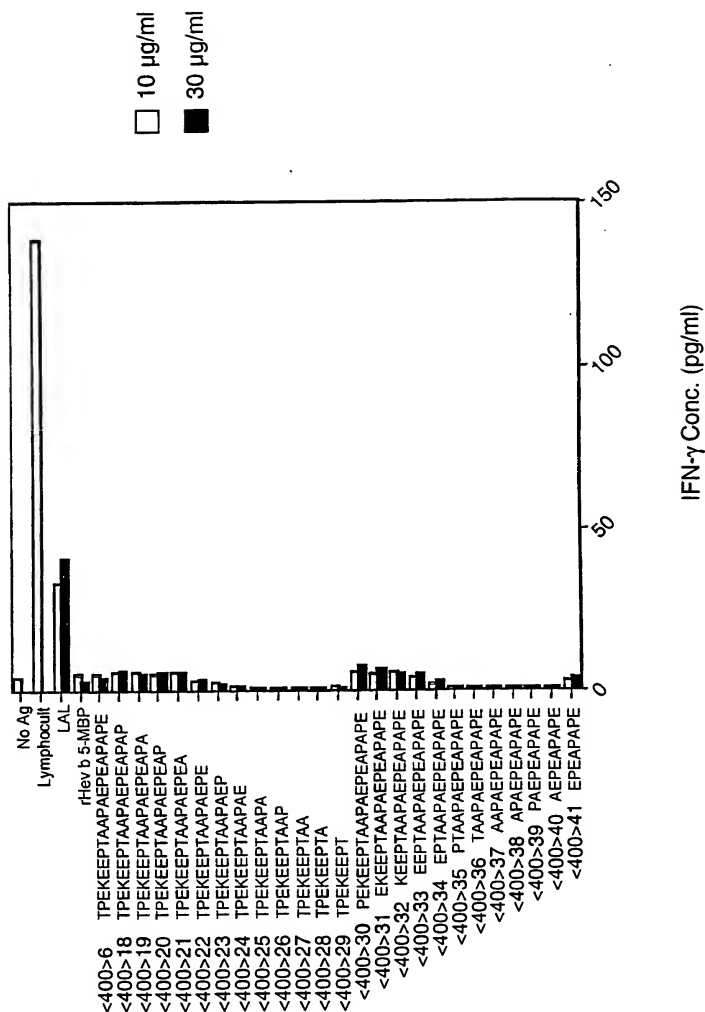
13/14



Minimal epitope: EPTAAPEAPE <400>42

Figure 10b

14/14



Minimal epitope: EPTAAPAEPE <400>42

- 1 -

SEQUENCE LISTING

<110> Monash University and Cooperative Research Centre for Asthma

<120> Immunointeractive Molecules and Uses Thereof

<130> 2339272/TDO

<140>

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<150> PQ3057/99

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/01182

A. CLASSIFICATION OF SUBJECT MATTER		
Int. Cl. ⁷ : C07K 14,725, 7/08, 16/28; C07H 21/04; A61K 38/10, 38/17, 39/395; A61P 37/08, 11/06		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
STN -DGENE sequence search SEQUENCE IDS 6, 7, 8, 14, 16, 42; ANGIS sequence search SEQUENCE ID 1, MEDLINE- Keyword search, -Keywords hev b , latex ,		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J.Biol.Chem. Volume 271, No: 41 Issued 11 October 1996. Slater et al " Identification, Cloning and Sequence of a Major Allergen (Hev b 5) from Natural Rubber Latex". Pages 25394-25399 and Genpept Accession No: Q39967, T10768, AAC49447 , See whole document	1, 12, 23-25, 27, 31-40
X	Molecular Immunology Volume 36 (1999) Slater et al "Murine B-cell and T-cell epitopes of the allergen Hev b 5 from natural rubber latex." Pages 135-143, See figures 1 and 2 in particular.	1-40
X	J. Allergy Clin.Immunol. Volume 103 No: 6. Issued June 1999. Beezhold et al "Human IgE-binding epitopes of the latex allergen Hev b5." Pages 1166-1172 See in particular discussion and figure 3.	1-40
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input type="checkbox"/> See patent family annex		
<p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p>		
Date of the actual completion of the international search 21 November 2000		Date of mailing of the 1 - DEC 2000
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustria.gov.au Facsimile No. (02) 6285 3929		Authorized officer K. LEVER Telephone No : (02) 6283 2254

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU00/01182

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	J. Allergy. Clin. Immunol. Volume 105, No: 5. Issued May 2000, De Silva et al. "Human T-cell epitopes of the latex allergen Hev b5 in Health care workers." Pages 1017-1024. See in particular figures 2 and 3 the discussion.	1-40
X	J. Biol. Chem. Volume 271, No: 41. Issued 11 October 1996. Akasawa et al. "A Novel Acidic Allergen, Hev b 5, in Latex." Pages 25389-25393. See document as a whole.	1, 12, 23-25, 27,31-40
Y		1, 12
Y	Plant Mol. Biol. Volume 25. No: 5. August 1994. Ledger et al. "Cloning and characterisation of five cDNAs for genes differentially expresses during fruit development of Kiwi fruit." Pages 877-886. Swiss-Prot Accession Number P43393.	1,12

PATENT COOPERATION TREATY

WEDNESDAY 11 APR 2001

From 
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT



To: Agent :

DAVIES COLLISON CAVE
1 Little Collins Street
MELBOURNE VIC 3000

**NOTIFICATION OF RECEIPT
OF DEMAND BY COMPETENT INTERNATIONAL
PRELIMINARY EXAMINING AUTHORITY**

(PCT Rule 59.3(e) and 61.1(b), first sentence
and Administrative Instructions, Section 601(a))

Date of mailing (day/month/year)	9 APR 2001 (9/4/01)
-------------------------------------	------------------------

Applicant's or agent's file reference
2339272

IMPORTANT NOTIFICATION

International application No.
PCT/AU00/01182

International filing date (day/month/year)
22 SEP 2000 (22/9/00)

Priority date (day/month/year)
24 SEP 1999 (24/9/99)

Applicant

Monash University (et al.)

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

4 APR 2001 (4/4/01)

2. That date of receipt is:



the actual date of receipt of the demand by this Authority (Rule 61.1(b)).



the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).



the date on which this Authority has, in response to the Invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. ☐ **Attention:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the elections(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide, Volume II*.



(If applicable) This notification confirms the information given by telephone, facsimile transmission or in person on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/AU
AUSTRALIAN PATENT OFFICE
PO BOX 200, WOTEN ACT 2606, AUSTRALIA
E-mail: pct@ipaaustralia.gov.au
Facsimile No. 02 6285 3929

Authorized officer

JOHN COLDWELL
02 6283 7924

Telephone No.

EA/ _____

PCT

CHAPTER II

DEMAND

Under Article 31 of the Patent Cooperation Treaty:

The Undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only _____

Identification of IPEA		Date of receipt of DEMAND
Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference 2339272/TDO
International application No. PCT/AU00/01182	International filing date (day/month/year) (22.09.2000) 22 September, 2000	(Earliest) Priority date (day/month/year) (24/09/1999) 24 September, 1999
Title of invention Immunointeractive molecules and uses thereof		
Box No. II APPLICANT(S)		
Name and address: (Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.) MONASH UNIVERSITY Wellington Road, Clayton 3168 Victoria, Australia		Telephone No.: Facsimile No.: Teleprinter No.:
State (that is, country) of nationality: Australia		State (that is, country) of residence: Australia
Name and address: (Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.) COOPERATIVE RESEARCH CENTRE FOR ASTHMA Level 8, Building 82 Royal Prince Alfred Hospital Camperdown 2050 New South Wales, Australia		
State (that is, country) of nationality: Australia		State (that is, country) of residence: Australia
Name and address: (Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.) O'HEHIR, Robyn 120 Gatehouse Street Parkville 3052 Victoria, Australia		
State (that is, country) of nationality: Australia		State (that is, country) of residence: Australia
<input type="checkbox"/> Further applicants are indicated on a continuation sheet.		

Box No. III AGENT OR COMMON REPRESENTATIVE: OR ADDRESS FOR CORRESPONDENCEThe following person is ☒ agent ☐ common representativeand ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.Name and address: (Family name followed by given name: for a legal entity, full official designation.
The address must include postal code and name of country.)HUGHES, E John L
SLATTERY, John M
CAINE, Michael JDAVIES COLLISON CAVE
1 Little Collins Street,
Melbourne 3000
Victoria, Australia

Telephone No.:

+61 3 9254 2777

Facsimile No.:

+61 3 9254 2770

Electronic Mail:

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.**Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION**

Statement concerning amendments: *

1. The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filedthe description ☐ as originally filed
☐ as amended under Article 34the claims ☐ as originally filed
☐ as amended under Article 19 (together with any accompanying statement)
☐ as amended under Article 34the drawings ☐ as originally filed
☐ as amended under Article 342. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69. 1(d)). *This check-box may be marked only where the time limit under Article 19 has not yet expired.*

- Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

- ☒ which is the language in which the international application was filed.
- ☐ which is the language of a translation furnished for the purposes of international search.
- ☐ which is the language of publication of the international application.
- ☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATESThe applicant hereby elects all eligible States (*that is, all States which have been designated and which are bound by Chapter II of the PCT*)

Excluding the following States which the applicant wishes not to elect:

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

For International Preliminary
Examining Authority use only

- | | | | |
|----|--|---|--------|
| 1. | translation of international application | : | sheets |
| 2. | amendments under Article 34 | : | sheets |
| 3. | copy (or, where required, translation) of
amendments under Article 19 | : | sheets |
| 4. | copy (or, where required, translation) of
statement under Article 19 | : | sheets |
| 5. | letter | : | sheets |
| 6. | other (specify) | : | sheets |

received	not received
----------	--------------

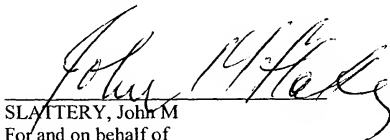
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

The demand is also accompanied by the item(s) marked below:

- | | |
|---|--|
| 1. <input type="checkbox"/> fee calculation sheet | 4. <input type="checkbox"/> statement explaining lack of signature |
| 2. <input type="checkbox"/> separate signed power of attorney | 5. <input type="checkbox"/> nucleotide and/or amino acid sequence listing in
computer readable form |
| 3. <input type="checkbox"/> copy of general power of attorney:
reference number, if any: | 6. <input type="checkbox"/> other (specify): |

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).


SLATTERY, John M
For and on behalf of
the applicant/s

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use

Demand received from IPEA on:

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE
COMMUNICATION OF THE INTERNATIONAL
APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

HUGHES, E., John, L.
Davies Collision Cave
1 Little Collins Street
Melbourne, VIC 3000
AUSTRALIE

WEDNESDAY, 18 APR 2001

Date of mailing (day/month/year) 05 April 2001 (05.04.01)		IMPORTANT NOTICE	
Applicant's or agent's file reference 2339272/TDO			
International application No. PCT/AU00/01182	International filing date (day/month/year) 22 September 2000 (22.09.00)	Priority date (day/month/year) 24 September 1999 (24.09.99)	
Applicant MONASH UNIVERSITY et al			

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
AU,KP,KR,US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
AE,AG,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,BZ,CA,CH,CN,CR,CU,CZ,DE,DK,DM,DZ,EA,EE,EP,ES,
FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,
MN,MW,MX,MZ,NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,
The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).
3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
05 April 2001 (05.04.01) under No. WO 01/23429

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

From the INTERNATIONAL SEARCHING AUTHORITY

To:

DAVIES COLLISON CAVE
1 Little Collins Street
MELBOURNE VIC 3000

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing 1 - DEC 2000
(day/month/year)

Applicant's or agent's file reference
2339272/EJH/TDO/LMa

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.

International filing date

PCT/AU00/01182

22 September 2000

Applicant

MONASH UNIVERSITY et al

1. ☒ The applicant is hereby notified that the international search report has been established and is transmitted herewith

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after 18 months from the priority date, the international application will be published by the International Bureau.

If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

Within 19 months from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later)

Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
E-mail address: pct@ipaustralia.gov.au
Facsimile No. (02) 6285 3929

Authorized officer

K. LEVER

Telephone No. (02) 6283 2254

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE
 in its capacity as elected Office

Date of mailing (day/month/year) 22 June 2001 (22.06.01)	
International application No. PCT/AU00/01182	Applicant's or agent's file reference 2339272/TDO
International filing date (day/month/year) 22 September 2000 (22.09.00)	Priority date (day/month/year) 24 September 1999 (24.09.99)
Applicant O'HEHIR, Robyn	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
04 April 2001 (04.04.01)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was

☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer P. Blanchet (Fax 338.87.40)
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

TUESDAY, 16 OCT 2001

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

DAVIES COLLISON CAVE
1 Little Collins Street
MELBOURNE VIC 3000

PCT

NOTIFICATION OF TRANSMITTAL OF
INTERNATIONAL PRELIMINARY EXAMINATION
REPORT

(PCT Rule 71.1)

Date of mailing
day/month/year

15 OCT 2001

Applicant's or agent's file reference
2339272

IMPORTANT NOTIFICATION

International Application No.

PCT/AU00/01182

International Filing Date

22 September 2000

Priority Date

24 September 1999

Applicant

MONASH UNIVERSITY et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translations to those Offices.
4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide

Name and mailing address of the IPEA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
E-mail address: pct@ipaustalia.gov.au
Facsimile No. (02) 6285 3929

Authorized officer

K. LEVER

Telephone No. (02) 6283 2254

ATENT COOPERATION TREA
PCT
INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2339272	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. PCT/AU00/01182	International Filing Date (<i>day/month/year</i>) 22 September 2000	Priority Date (<i>day/month/year</i>) 24 September 1999
International Patent Classification (IPC) or national classification and IPC Int. Cl. ⁷ C07K 14/725, 7/08, 16/28; C07H 21/04; A61K 38/10, 39/395; A61P 37/08, 11/06		
Applicant MONASH UNIVERSITY et al		

1.	This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.																								
2.	This REPORT consists of a total of 5 sheets, including this cover sheet. <input type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT). These annexes consist of a total of sheet(s).																								
3.	This report contains indications relating to the following items: <table><tr><td>I</td><td><input checked="" type="checkbox"/></td><td>Basis of the report</td></tr><tr><td>II</td><td><input type="checkbox"/></td><td>Priority</td></tr><tr><td>III</td><td><input type="checkbox"/></td><td>Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td></tr><tr><td>IV</td><td><input type="checkbox"/></td><td>Lack of unity of invention</td></tr><tr><td>V</td><td><input checked="" type="checkbox"/></td><td>Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td></tr><tr><td>VI</td><td><input type="checkbox"/></td><td>Certain documents cited</td></tr><tr><td>VII</td><td><input type="checkbox"/></td><td>Certain defects in the international application</td></tr><tr><td>VIII</td><td><input checked="" type="checkbox"/></td><td>Certain observations on the international application</td></tr></table>	I	<input checked="" type="checkbox"/>	Basis of the report	II	<input type="checkbox"/>	Priority	III	<input type="checkbox"/>	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	IV	<input type="checkbox"/>	Lack of unity of invention	V	<input checked="" type="checkbox"/>	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	VI	<input type="checkbox"/>	Certain documents cited	VII	<input type="checkbox"/>	Certain defects in the international application	VIII	<input checked="" type="checkbox"/>	Certain observations on the international application
I	<input checked="" type="checkbox"/>	Basis of the report																							
II	<input type="checkbox"/>	Priority																							
III	<input type="checkbox"/>	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability																							
IV	<input type="checkbox"/>	Lack of unity of invention																							
V	<input checked="" type="checkbox"/>	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement																							
VI	<input type="checkbox"/>	Certain documents cited																							
VII	<input type="checkbox"/>	Certain defects in the international application																							
VIII	<input checked="" type="checkbox"/>	Certain observations on the international application																							

Date of submission of the demand 4 April 2001	Date of completion of the report 8 October 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer K. LEVER Telephone No. (02) 6283 2254

I. Basis of the report

1. With regard to the elements of the international application:*
- ☒ the international application as originally filed.
- ☐ the description, pages , as originally filed,
 pages , filed with the demand,
 pages , received on with the letter of
- ☐ the claims, pages , as originally filed,
 pages , as amended (together with any statement) under Article 19,
 pages , filed with the demand,
 pages , received on with the letter of
- ☐ the drawings, pages , as originally filed,
 pages , filed with the demand,
 pages , received on with the letter of
- ☐ the sequence listing part of the description:
 pages , as originally filed
 pages , filed with the demand
 pages , received on with the letter of
2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:
- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).
3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:
- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished
4. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/fig.
5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**
- * Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).
- ** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report

V.	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement																				
1.	<p data-bbox="164 171 239 192">Statement</p> <table border="0"> <tr> <td data-bbox="164 205 404 226">Novelty (N)</td> <td data-bbox="404 205 851 226">Claims</td> <td data-bbox="851 205 1003 226">YES</td> </tr> <tr> <td></td> <td data-bbox="404 240 851 260">Claims 1-40</td> <td data-bbox="851 240 1003 260">NO</td> </tr> <tr> <td data-bbox="164 274 404 294">Inventive step (IS)</td> <td data-bbox="404 274 851 294">Claims</td> <td data-bbox="851 274 1003 294">YES</td> </tr> <tr> <td></td> <td data-bbox="404 308 851 329">Claims 1-40</td> <td data-bbox="851 308 1003 329">NO</td> </tr> <tr> <td data-bbox="164 343 404 363">Industrial applicability (IA)</td> <td data-bbox="404 343 851 363">Claims 1-40</td> <td data-bbox="851 343 1003 363">YES</td> </tr> <tr> <td></td> <td data-bbox="404 377 851 397">Claims</td> <td data-bbox="851 377 1003 397">NO</td> </tr> </table>			Novelty (N)	Claims	YES		Claims 1-40	NO	Inventive step (IS)	Claims	YES		Claims 1-40	NO	Industrial applicability (IA)	Claims 1-40	YES		Claims	NO
Novelty (N)	Claims	YES																			
	Claims 1-40	NO																			
Inventive step (IS)	Claims	YES																			
	Claims 1-40	NO																			
Industrial applicability (IA)	Claims 1-40	YES																			
	Claims	NO																			
2.	<p data-bbox="164 418 430 439">Citations and explanations (Rule 70.7)</p> <p data-bbox="164 459 760 480">J.Biol. Chem. Volume 271, No 41 issued October 11 1996 pages 25394-25399.</p> <p data-bbox="164 487 1003 528">This document discloses a hev b5 sequence identical to sequence 1 of the current application. Therefore claim 1 can not be considered novel when X₂ is this sequence and X₁ and X₃ are 0 amino acid residues.</p> <p data-bbox="164 541 728 562">The document also discloses that this sequence elicits an allergic response.</p> <p data-bbox="164 569 1003 637">Claim 12 is considered broader than claim 1 because it does not limit the sequence in any way other than to be derived from or homologous to hev b5 and this document impacts on the novelty of this claim in the same way as claim 1.</p> <p data-bbox="164 644 1003 706">Claims 23, 24, 25 and 27 are considered not novel nor inventive in light of this document because it discusses monoclonal antibodies and it is also well within the person skilled in the art ability to produce them once they have the sequence.</p> <p data-bbox="164 713 1003 823">Claims 31 -40 are considered not inventive in relation to this document because it is well within the skills of the person skilled in the art to produce and isolate a nucleic acid sequence after finding the peptide. The invention as defined in claims 32-40 is not specifically disclosed or exemplified in the cited document but there is enough of an indication that the person skilled in the art would follow this pathway without any inventive faculty.</p> <p data-bbox="164 830 1003 871">Claims 2-11 are considered novel over this document because they are restricted to the shorter peptides which are not contemplated in this document.</p> <p data-bbox="164 919 601 939">Molecular Immunology volume 36 (1999) pages 135-143.</p> <p data-bbox="164 946 1003 1008">This document discloses the hev b5 sequence which impacts on the novelty of claim 1 for the same reasons as explained above. The document also discloses shorter peptides Figure 1 disclosing Octapeptides of hev b5 and Fig 2 20mer peptides of hev b5. Thus claims 2-11 are considered not novel or inventive.</p> <p data-bbox="164 1022 1003 1063">The DNA sequence of hev b5 was cloned and expressed indicating that the nucleotide sequence was known this impacts on the novelty of claim 31.</p> <p data-bbox="164 1070 1003 1111">Both B-cell and T-cell epitopes were detected. The use of antibodies to detect B-cells epitopes is disclosed and the use of the shorter peptides to find the exact position of the epitopes is disclosed</p> <p data-bbox="164 1118 1003 1159">It is therefore considered that in light of this document claims 1-40 are not considered novel and they lack an inventive step.</p> <p data-bbox="728 1173 984 1193" style="text-align: right;">Continued in Supplemental Box.</p>																				

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 26, 28 and 30 refer to antibodies 1C10, 6F6, 3G3 and 6A10. These claims are not fully supported by the description. It appears these are monoclonal antibodies and as such the description should contain sequence information relating to them, how to produce them, and/or information on where these antibodies have been deposited.

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of V2**Citations and Explanations:**

Journal of Allergy and Clinical Immunology, Volume 103, pages 1166-1172, (1999)

This document discloses the whole hev b5 sequence and the smaller octapeptide sequences.

T-cell epitopes and the peptides containing them are discussed as being used for successfully lowering IgE levels in sensitised patients.

Claims 1-40 are considered not novel and lack an inventive step over this document for similar reasons as explained for the previous documents.

Journal of Allergy Clin. Immunol. Volume 105, No: 5. Issued May 2000

This document was published after the priority date of the application and would only impact on novelty if the current claims were at a later point found not to be entitled to this priority.

This document discusses the smaller 20mer peptides which span the whole length of hev b5. Results indicate where the T-cell epitopes lie in the hev b5 sequence

J. Biol. Chem. Volume 271 No: 41 issued 11 October 1996.

This document discusses the hev b5 sequence and its role in latex allergies. It also discusses the similarity between Kiwifruit and hev b5 and their relationship to IgE's. It does not discuss the small peptides so is most relevant to claim 1 and the later use claims. Claims 1, 12, 23-25, 27 and 31-40 are considered not novel and not to possess an inventive step in light of this document.

Plant. Mol. Biol. Volume 25 pages 877-886 (1994)

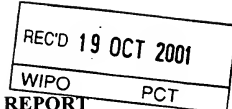
This document is relevant to claims 1 and 12 when combined with *J. Biol. Chem.* Volume 271. No: 41.

It discloses the kiwifruit whole sequence which would be excluded from claim 1 because it has a length of 180 amino acids. But the person skilled in the art having read and understood *J. Biol. Chem.* in regard to the latex and the kiwifruit allergies being similar, the sequence homology between hev b5 and pKIWI501 is 46% overall with particular sections having homologies as high as 80%, and eliciting the same T-cell mediation would attempt find the exact epitopes that cause these reactions. Therefore claims 1 and 12 are considered not to contain an inventive step over these combined documents.

It is considered that claims 1-40 have Industrial applicability.

PATENT COOPERATION TREATY
PCT
INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)



Applicant's or agent's file reference 2339272	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. PCT/AU00/01182	International Filing Date (day/month/year) 22 September 2000	Priority Date (day/month/year) 24 September 1999
International Patent Classification (IPC) or national classification and IPC Int. Cl.⁷ C07K 14/725, 7/08, 16/28; C07H 21/04; A61K 38/10, 39/395; A61P 37/08, 11/06		
Applicant MONASH UNIVERSITY et al		

1.	This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2.	This REPORT consists of a total of 5 sheets, including this cover sheet. <input type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT). These annexes consist of a total of sheet(s).
3.	This report contains indications relating to the following items: I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application

Date of submission of the demand 4 April 2001	Date of completion of the report 8 October 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer K. LEVER Telephone No. (02) 6283 2254

I. Basis of the report**1. With regard to the elements of the international application:***

- ☒ the international application as originally filed.
- ☐ the description, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of
- ☐ the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , received on with the letter of
- ☐ the drawings, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of
- ☐ the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	YES
	Claims 1-40	NO
Inventive step (IS)	Claims	YES
	Claims 1-40	NO
Industrial applicability (IA)	Claims 1-40	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

J.Biol. Chem. Volume 271, No 41 issued October 11 1996 pages 25394-25399.

This document discloses a hev b5 sequence identical to sequence 1 of the current application. Therefore claim 1 can not be considered novel when X_2 is this sequence and X_1 and X_3 are 0 amino acid residues.

The document also discloses that this sequence elicits an allergic response.

Claim 12 is considered broader than claim 1 because it does not limit the sequence in any way other than to be derived from or homologous to hev b5 and this document impacts on the novelty of this claim in the same way as claim 1.

Claims 23, 24, 25 and 27 are considered not novel nor inventive in light of this document because it discusses monoclonal antibodies and it is also well within the person skilled in the art ability to produce them once they have the sequence.

Claims 31-40 are considered not inventive in relation to this document because it is well within the skills of the person skilled in the art to produce and isolate a nucleic acid sequence after finding the peptide. The invention as defined in claims 32-40 is not specifically disclosed or exemplified in the cited document but there is enough of an indication that the person skilled in the art would follow this pathway without any inventive faculty.

Claims 2-11 are considered novel over this document because they are restricted to the shorter peptides which are not contemplated in this document.

Molecular Immunology volume 36 (1999) pages 135-143.

This document discloses the hev b5 sequence which impacts on the novelty of claim 1 for the same reasons as explained above. The document also discloses shorter peptides Figure 1 disclosing Octapeptides of hev b5 and Fig 2 20mer peptides of hev b5. Thus claims 2-11 are considered not novel or inventive.

The DNA sequence of hev b5 was cloned and expressed indicating that the nucleotide sequence was known this impacts on the novelty of claim 31.

Both B-cell and T-cell epitopes were detected. The use of antibodies to detect B-cells epitopes is disclosed and the use of the shorter peptides to find the exact position of the epitopes is disclosed

It is therefore considered that in light of this document claims 1-40 are not considered novel and they lack an inventive step.

Continued in Supplemental Box.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 26, 28 and 30 refer to antibodies 1C10, 6F6, 3G3 and 6A10. These claims are not fully supported by the description. It appears these are monoclonal antibodies and as such the description should contain sequence information relating to them, how to produce them, and/or information on where these antibodies have been deposited.

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of V2**Citations and Explanations:**

Journal of Allergy and Clinical Immunology, Volume 103, pages 1166-1172, (1999)

This document discloses the whole hev b5 sequence and the smaller octapeptide sequences.

T-cell epitopes and the peptides containing them are discussed as being used for successfully lowering IgE levels in sensitised patients.

Claims 1-40 are considered not novel and lack an inventive step over this document for similar reasons as explained for the previous documents.

Journal of Allergy Clin. Immunol. Volume 105, No: 5. Issued May 2000

This document was published after the priority date of the application and would only impact on novelty if the current claims were at a later point found not to be entitled to this priority.

This document discusses the smaller 20mer peptides which span the whole length of hev b5. Results indicate where the T-cell epitopes lie in the hev b5 sequence

J. Biol. Chem. Volume 271 No: 41 issued 11 October 1996.

This document discusses the hev b5 sequence and its role in latex allergies. It also discusses the similarity between Kiwifruit and hev b5 and their relationship to IgE's. It does not discuss the small peptides so is most relevant to claim 1 and the later use claims. Claims 1, 12, 23-25, 27 and 31-40 are considered not novel and not to possess an inventive step in light of this document.

Plant. Mol. Biol. Volume 25 pages 877-886 (1994)

This document is relevant to claims 1 and 12 when combined with J. Biol. Chem. Volume 271. No: 41.

It discloses the kiwifruit whole sequence which would be excluded from claim 1 because it has a length of 180 amino acids. But the person skilled in the art having read and understood J. Biol. Chem in regard to the latex and the kiwifruit allergies being similar, the sequence homology between hev b5 and pKIWI501 is 46% overall with particular sections having homologies as high as 80%, and eliciting the same T-cell mediation would attempt find the exact epitopes that cause these reactions. Therefore claims 1 and 12 are considered not to contain an inventive step over these combined documents.

It is considered that claims 1-40 have Industrial applicability.



INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 2339272/EJH/TDO/LMa	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/AU00/01182	International filing date (day/month/year) 22 September 2000	(Earliest) Priority Date (day/month/year) 24 September 1999
Applicant MONASH UNIVERSITY et al		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (See Box II).

4. With regard to the title, ☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

Immunointeractive molecules and their uses in treating subjects suffering from Hev b 5 Allergies.

5. With regard to the abstract, ☒ the text is approved as submitted by the applicant

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure

☐ because this figure better characterizes the invention

☒ None of the figures

A. CLASSIFICATION OF SUBJECT MATTER		
Int. Cl. ⁷ : C07K 14,725, 7/08, 16/28; C07H 21/04; A61K 38/10, 38/17, 39/395; A61P 37/08, 11/06		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) STN -DGENE sequence search SEQUENCE IDS 6, 7, 8, 14, 16, 42; ANGIS sequence search SEQUENCE ID 1, MEDLINE- Keyword search, -Keywords hev b , latex ,		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J.Biol.Chem. Volume 271, No: 41 issued 11 October 1996. Slater et al "Identification, Cloning and Sequence of a Major Allergen (Hev b 5) from Natural Rubber Latex". Pages 25394-25399 and Genpept Accession No: Q39967, T10768, AAC49447 , See whole document	1, 12, 23-25, 27, 31-40
X	Molecular Immunology Volume 36 (1999) Slater et al "Murine B-cell and T-cell epitopes of the allergen Hev b 5 from natural rubber latex." Pages 135-143, See figures 1 and 2 in particular.	1-40
X	J. Allergy Clin.Immunol. Volume 103 No: 6. Issued June 1999. Beezhold et al "Human IgE-binding epitopes of the latex allergen Hev b5." Pages 1166-1172 See in particular discussion and figure 3.	1-40
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input type="checkbox"/> See patent family annex		
* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family	
"A" document defining the general state of the art which is not considered to be of particular relevance		
"E" earlier application or patent but published on or after the international filing date		
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 21 November 2000	Date of mailing of the international search report 1 - DEC 2000	
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. (02) 6285 3929	Authorized officer K. LEVER Telephone No : (02) 6283 2254	

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	J. Allergy. Clin. Immunol. Volume 105, No: 5. Issued May 2000, De Silva et al. "Human T-cell epitopes of the latex allergen Hev b5 in Health care workers." Pages 1017-1024. See in particular figures 2 and 3 the discussion. /	1-40
X	J. Biol. Chem. Volume 271, No: 41. Issued 11 October 1996. Akasawa et al. "A Novel Acidic Allergen, Hev b 5, in Latex." Pages 25389-25393. See document as a whole. /	1, 12, 23-25, 27,31-40
Y		1, 12
Y	Plant Mol. Biol. Volume 25. No: 5. August 1994. Ledger et al. "Cloning and characterisation of five cDNAs for genes differentially expresses during fruit development of Kiwi fruit." Pages 877-886. Swiss-Prot Accession Number P43393. /	1,12